

THE EPIZOOTIOLOGY AND PATHOGENICITY OF
Haemoproteus meleagridis Levine, 1961,
FROM FLORIDA TURKEYS

BY

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To Mom and Dad

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Abstract of Dissertation Presented to the Graduate School
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THE EPIZOOTIOLOGY AND PATHOGENICITY OF
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FROM FLORIDA TURKEYS

By

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Avian species of the genus Haemoproteus are common haemosporidian parasites found in many families of birds. In spite of their widespread occurrence, little is known of their vectors, epizootiology and pathogenicity.

Haemoproteus meleagridis occurs in Wild Turkeys throughout the southeastern United States. Results of this study showed that members of at least 5 species of ceratopogonid flies in the genus Culicoides, i.e. Culicoides edeni, Culicoides hinmani, Culicoides arboricola, Culicoides knowltoni and Culicoides haematopotus, could support development of the sporogonic stages of the parasite.

Results of a 2-year epizootiological study of Haemoproteus meleagridis indicated that Culicoides edeni is

the most important vector in Florida. Conclusions were based on the high susceptibility of Culicoides edeni to the parasite, its preponderance in biting collections, its activity and vertical distribution in the forest canopy and on isolations of Haemoproteus meleagridis from naturally infected specimens. Results of a concurrent sentinel study indicated that transmission of the parasite occurred year-round in southern Florida. The prevalence of transmission in the more temperate climate of northern Florida was lower, more variable and limited to between April and December.

Sporozoite-induced experimental infections produced a moderate to severe myositis in young domestic turkeys and had significant effects on their growth and weight gain. Pathological effects were associated with the development of megaloschizonts in skeletal and cardiac muscle. Megaloschizonts had a thick, hyaline wall, were aseptate and were morphologically similar to those of Haemoproteus desseri and Arthrocystis galli.

At least 2 generations of schizogony occurred. First-generation schizonts matured between 5 and 8 days post-infection and produced elongate merozoites. Second-generation megaloschizonts developed between 8 and 17 days post-infection and yielded spherical merozoites that developed to form erythrocytic gametocytes.

Haemoproteus meleagridis was transmitted experimentally to a Chuckar and a Ring-necked Pheasant, but not to chickens, Guineafowl or Northern Bobwhites.

The fine structure of circulating and exflagellating gametocytes was similar to that of other avian haemoproteids. The fine structure and development of oocysts was similar to oocysts of species of Leucocytozoon. Mature megaloschizonts differed ultrastructurally from similar forms reported from species of Leucocytozoon.

INTRODUCTION

The protozoan genus Haemoproteus is composed of over 130 different species of blood and tissue parasites of birds and some reptiles (Levine and Campbell, 1971; Bennett et al., 1982). Since the discovery of these organisms by Danilewsky (1889) nearly a century ago, little beyond the basic alpha-taxonomy of this group has been studied. This is primarily because laboratory studies of these species are difficult to accomplish. Transmission of avian haemoproteids by blood inoculation is rarely successful because the parasites do not undergo asexual schizogony in circulating erythrocytes. Attempts by a number of workers to transmit the parasites using tissue homogenates or transplants containing the exoerythrocytic stages have occasionally succeeded, but have not been reliable enough for practical use (Gondor, 1915; O'Roke, 1930; Coatney, 1933; Lastra and Coatney, 1950; Bierer et al., 1959).

Another major obstacle to the study of this genus has been the absence of a convenient laboratory model. Most studies of the life cycle, fine structure and

physiology of avian haemoproteids have been limited to the few species that infect columbiformes, i.e. H. columbae, H. sacharovi, H. maccallumi and H. palumbis. Pigeons and doves are relatively inexpensive, easy to breed in captivity and can produce a reliable, although limited, supply of uninfected young for experimental work. The early discovery that at least 4 different species of ectoparasitic hippoboscids flies could transmit H. columbae (Sergeant and Sergeant, 1906; Arāgao, 1908, 1916; Gondor, 1915) led to a number of classical studies of its life cycle during the first half of this century (Acton and Knowles, 1914; Adie, 1915, 1925; Coatney, 1933). Further work has been dampened by the difficulty in rearing hippoboscids in captivity and harvesting large numbers of sporozoites for experimental infections.

With the discovery that ceratopogonids in the genus Culicoides could support development of the sporogonic stages of Haemoproteus nettionis from wild anatids, a potential laboratory model using domestic ducks became available (Fallis and Wood, 1957). Unfortunately, Culicoides are notoriously difficult to colonize and less available than hippoboscids for experimental work. Miltgen et al. (1981) were able to obtain sporozoites of H. desseri from Blossom-Headed Parakeets, Psittacula roseata, from Southeast Asia by exposing infected birds to a colony

of C. nubeculosus, but neither the avian host nor the vector are available to workers in this country.

Currently, vectors have been reported for fewer than 10% of the described species of Haemoproteus (Table 1). Five of these, i.e. H. columbae, H. sacharovi, H. maccallumi, H. lophortyx and H. palumbis, have been transmitted by the bite of hippoboscids flies. The remaining 7 species are presumed to be transmitted by ceratopogonid flies in the genus Culicoides. Several workers have reported complete development of the sporogonic stages in 8 species of Culicoides and have transmitted the parasite by intraperitoneal or intravenous inoculation of sporozoites into suitable, uninfected hosts (Bennett et al., 1965; Kahn and Fallis, 1971; Miltgen et al, 1981; Atkinson et al., 1983). Transmission by bite has not been demonstrated for any haemoproteid in this group.

The discovery that hippoboscids flies could transmit Haemoproteus, their common occurrence on birds and the morphological similarities among gametocytes of various species, led to the early assumption that this group was fairly homogeneous. The recent advances in understanding of the complex life cycles of cyst forming coccidian parasites such as Sarcocystis and Toxoplasma, has demonstrated that sporozoan life cycles are far more

Table 1: Proven and presumed vectors of avian haemoproteids

Species	Vector	Author
<u>H. columbae</u>	<u>Pseudolynchia canariensis</u>	Sergent and Sergent, 1906
	<u>P. brunnea</u>	Arãgao, 1908
	<u>P. capensis</u>	Gonder, 1915
	<u>Microlynchia pusilla</u>	Arãgao, 1916
<u>H. sacharovi</u>	<u>P. canariensis</u>	Huff, 1932
<u>H. maccallurni</u>	<u>P. canariensis</u>	Huff, 1932
<u>H. lophortyx</u>	<u>Stilbometopa impressa</u>	O'Roke, 1930
	<u>Lynchia hirsuta</u>	Tarshis, 1955
<u>H. palumbis</u>	<u>Ornithomyia avicubria</u>	Baker, 1966b
<u>H. desseri</u>	<u>Culicoides nubeculosus</u>	Miltgen et al., 1981
<u>H. nettionis</u>	<u>C. downesi</u>	Fallis and Wood, 1957
<u>H. velans</u>	<u>C. stilobezziodes</u>	Kahn and Fallis, 1971
	<u>C. sphagnumensis</u>	Kahn and Fallis, 1971
<u>H. canachites</u>	<u>C. sphagnumensis</u>	Fallis and Bennett, 1960
<u>H. fringillae</u>	<u>C. crepuscularis</u> *	Fallis and Bennett, 1961
	<u>C. stilobezziodes</u> *	Fallis and Bennett, 1961
<u>H. danilewskyi</u>	<u>C. crepuscularis</u> *	Bennett and Fallis, 1960
	<u>C. stilobezziodes</u> *	Bennett and Fallis, 1960
	<u>C. sphagnumensis</u> *	Fallis and Bennett, 1961
<u>H. meleagridis</u>	<u>C. edeni</u>	Atkinson et al., 1983
	<u>C. hinmani</u>	Atkinson et al., 1983
	<u>C. arboricola</u>	Atkinson et al., 1983

* Transmission not carried out, but able to support development of oocysts and sporozoites.

diverse than was realized previously. There is no reason to suggest that haemoproteids are any less so. Accordingly, several authors have proposed that this genus should be divided into 2 genera (Bennett et al., 1965). They suggested that species transmitted by hippoboscid flies, with large oocysts containing several hundred blunt sporozoites, would remain in the genus Haemoproteus. Those species transmitted by ceratopogonids, with small oocysts containing fewer than 100 pointed sporozoites, would be placed in the genus Parahaemoproteus.

The size and presence or absence of septa in the exoerythrocytic schizonts has been proposed as another criterion for separating the 2 genera (Garnham, 1966). One form of schizont, found in birds infected with H. columbae, H. lophortyx, H. palumbis and H. fringillae, is sausage or oval shaped, lacks cytomeres, and occurs in endothelial cells in a variety of tissues including lung, liver, spleen, kidney, bone marrow, cecum and heart. The other type, described in birds infected with H. garnhami, is large and focal with diameters of 200 um or larger and contains numerous cytomeres, separated from one another by septa (Garnham, 1966). Unfortunately, schizont morphology has not correlated perfectly with vector or oocyst size. This is exemplified by the exoerythrocytic stages of H. fringillae (Khan and Fallis, 1969). This species, which develops in Culicoides, has

exoerythrocytic schizonts that lack cytomeres, as is true of the species transmitted by hippoboscid flies.

The proposed revisions in the classification have not found wide acceptance because the gametocytes (i.e. the main diagnostic stage) of each group cannot be distinguished. While the revision may be a more accurate reflection of phylogenetic relationships, the proposed reclassification is speculative and essentially nonfunctional at our current level of understanding. Until the life cycles of more species are studied, most workers have, by de facto consensus, relegated these 2 groups to subgeneric status.

Epizootiology

Since the vectors of most species of Haemoproteus are unknown, fundamental questions concerning their epizootiology, host specificity and pathogenicity have remained unanswered. Only a few studies have examined the seasonal transmission and role that various biting arthropods may play in the epizootiology of these parasites. In a study of Haemoproteus in insular Newfoundland, Bennett and Coombs (1975) did not find ookinetes, oocysts or sporozoites in 101 Ornithomyia fringillina recovered from passerine birds infected with

H. fallisi, H. fringillae or H. orizivora. They found sporozoites in 13.8% of 184 Culicoides stilobezziodes captured in bird baited traps and suggested that it was the sole vector. Later work by Greiner et al. (1978) demonstrated that a number of other ornithophilic Culicoides were present in their study area. It is possible that they may also contribute to transmission of the parasites.

Bennett and Fallis (1960) found high prevalences and high parasitemias of Haemoproteus in resident and migratory birds examined in June and July in Algonquin Park, Canada, when Culicoides populations were high. A preponderance of low level, chronic infections occurred during August and September when hippoboscids were abundant. Bennett (1960) captured 6 different species of Culicoides with traps baited with woodland and water birds in the same area and noted differences in distribution of the flies by habitat. Some species, i.e. C. stilobezziodes, C. sphagnumensis, C. crepuscularis and C. haematopotus, preferred the middle levels of the forest canopy, while others, i.e. C. downesi, preferred the lake shore. Bennett (1960) also found differences when comparisons were made by host. Culicoides downesi preferred ducks and herons to woodland birds as a blood source. He noted that both of these characteristics may be important in determining the types of blood parasites

found in certain species of birds as well as the specificity of the parasites themselves.

Anomalies in the prevalence of columbiform haemoproteids have led a number of other authors to question the importance of hippoboscids as vectors. Huff (1932), Hanson et al. (1957) and Greiner (1975) felt that the low prevalence of hippoboscids on columbiform birds at times when the incidence of Haemoproteus is high suggested the involvement of another vector. Greiner (1975) collected C. haematopotus and C. crepuscularis feeding on Mourning Doves, Zenaida macroura, in Nebraska during periods of active transmission of Haemoproteus. He hypothesized that they may play important roles as vectors of H. maccallumi and H. sacharovi. By contrast, other workers have found a close correlation between the seasonal occurrence of H. columbae and its hippoboscid vector, Pseudolynchia canariensis, in populations of Rock Doves, Columba livia, in Michigan (Klei and DeGiusti, 1975). Ayala et al. (1977) found large numbers of Microlynchia pusilla and Stilbometopa podostyla on Columbian Eared Doves, Zenaida auriculata, and felt that these hippoboscids could account alone for the high levels of transmission of H. maccallumi they observed throughout the year. Unfortunately, critical experiments to test whether columbiform haemoproteids can be transmitted by species of Culicoides have not been done.

Pathogenicity

Since the first detailed studies of Haemoproteus in the early part of this century, many authors have reported instances where this organism appeared to debilitate the host. Acton and Knowles (1914), Adie (1924), Coatney (1933) and Markus and Oosthuizen (1972) each observed a pigeon, heavily infected with H. columbae, that seemed weak, anemic and with a poor appetite. Wasielewski and Wülker (1918) reported 6 fatal infections of Haemoproteus in the thousand they examined and Becker et al. (1956) attributed the enlarged, purplish livers they found in domestic pigeon squabs to infections by H. sacharovi. Many workers have speculated that H. columbae and other avian haemoproteids must be pathogenic to some extent because peak parasitemias may involve half of the circulating erythrocytes (Levine, 1961; Garnham, 1966). Yet, there have been no experimental attempts to measure pathological changes in infected birds, even among columbiform hosts that can be infected fairly easily in the laboratory.

Only 1 study has attempted to document the pathogenicity of Haemoproteus in detail. O'Roke (1930) found that California Quail, Lophortyx californica, infected with H. lophortyx had variable amounts of pigment deposited in their lungs, testes, spleens and livers.

The infected birds tended to have lighter body weights, enlarged, blackened spleens and livers that were slightly smaller than normal. O'Roke (1930) felt that the parasitized blood cells had less oxygen carrying capacity and were less elastic and more likely to rupture when passing through small capillaries. He described 4 stages of disease in birds infected with H. lophortyx: 1) mild-chronic with no obvious signs of infection, 2) mild-acute where birds were restless and had poor appetites for 2 to 4 days, 3) moderate-chronic where birds were anemic, weak and more susceptible to death by exposure and exhaustion and 4) heavy-acute where birds lost weight, refused food, were unable to fly and eventually died. O'Roke commonly observed birds with moderate-chronic infections in the field, but saw only 4 heavy-acute infections in the several hundred birds he examined. Unfortunately, O'Roke did not attempt to reproduce the disease in experimentally infected quail or precisely quantify parasitemias and course of infection as they related to the stage of the disease.

More recently, Julian and Galt (1980) reported several incidents of a pathogenic Haemoproteus infection in Muscovy Ducks, Cairina moschata, from Ontario. They found large numbers of schizonts, morphologically similar to those of other species of Haemoproteus, in endothelial cells from a variety of tissues. The schizonts appeared to

cause vascular congestion and edema by mechanical interference with the circulation. In spite of the large number of exoerythrocytic parasites, infected muscovy ducks never developed patent infections with erythrocytic gametocytes. Yet, inoculation of blood from white Pekin ducks with patent H. nettionis infections reproduced the disease in uninfected muscovy ducks. Julian and Galt suggested that transmission occurred because inoculated blood contained a few exoerythrocytic merozoites. Later work by Sibley and Werner (1984) was unable to confirm the observations made by Julian and Galt (1980). They succeeded in transmitting H. nettionis to muscovy ducks using sporozoites from pools of naturally infected C. downsi. They failed to observe any pathological effects from the Haemoproteus infections. Julian et al. (1985) have since identified the pathogenic organism as an intracellular bacterium.

Host Specificity

The present classification of avian haemoproteids separates species with morphologically similar gametocytes by host family. Since morphologically identical species may occur in the same habitat where their hosts are exposed to the same vectors, it is possible that many species

will need to be synonymized once their life cycles are better known. Few of the experiments needed to confirm the current classification have been done. Huff (1932), Coatney (1933) and Baker (1957, 1966, 1968) studied the host specificity of H. sacharovi and H. maccallumi from Mourning Doves, Zenaidura macroura carolinensis, H. columbae from Rock Doves, Columba livia, and H. palumbus from Wood Pigeons, Columba palumbus, respectively. Huff (1932) was able to transmit H. sacharovi and H. maccallumi to Rock Doves by the bite of infected hippoboscids, but Coatney (1933) was unable to infect Mourning Doves with H. columbae by either fly bite or inoculation of sporozoites. Baker (1966b, 1968) attempted unsuccessfully to transmit H. palumbus from Wood Pigeons to Rock Doves by injection of sporozoites. Similar host restriction was demonstrated by Fallis and Bennett (1960) for H. canachites from a Spruce Grouse, Canachites canadensis. They inoculated sporozoites obtained from Culicoides sphagnumensis into uninfected Ruffed Grouse, Bonasa umbellus, domestic ducks, Anas boschas, a Rock Dove, Columba livia, and a Java Sparrow, Padra oryzivora. Only Ruffed Grouse became infected. These results must be interpreted with care since the authors did not include positive controls when they inoculated the Rock Dove and Java Sparrow.

Miltgen et al. (1981) unsuccessfully attempted to infect a parakeet, Melopsittacus sp., with H. desseri from Blossom-Headed Parakeets by intraperitoneal inoculation of sporozoites obtained from C. nubeculosus.

Fine Structure

For the most part, ultrastructural studies of the genus Haemoproteus have been limited to the mature and exflagellating gametocytes of H. columbae. Work by Bradbury and Roberts (1970), Bradbury and Trager (1968a, 1968b), Gallucci (1974a, 1974b) and Sterling and Aikawa (1973) has demonstrated that the morphology of these stages is consistent with that of other haemosporidians.

Studies of the ookinetes of H. columbae and H. velans have shown that they are similar to each other in structure and organization, but with several important differences. Gallucci (1974b) hypothesized that the ookinete is a conservative stage in the life cycle of the parasite, since it is produced sexually and more likely to retain the organelles of its primitive ancestor. Hence, differences between the ookinetes of these 2 species may lend credence to the idea that haemoproteids transmitted by Culicoides belong in a separate taxonomic group.

Desser (1972a, 1972b) suggested that the large crystalloid inclusions in ookinetes of H. velans originated from amorphous, dense lipid inclusions in the macrogametocyte. He felt that this material was converted to a crystalline form by addition of a protein component by a network of endoplasmic reticulum that surrounded the precursor material. In contrast, crystalloid particles in ookinetes of H. columbae first appeared between the lamellae of the endoplasmic reticulum (Gallucci, 1974b). Other differences between the ookinetes of these 2 species occur in the fine structure of their anterior ends. Gallucci (1974b) found a distinctive conoid in ookinetes of H. columbae. Ookinetes of other haemosporidia, including H. velans, seem to lack this structure, although observations among some species are limited (Gallucci, 1974b; Desser, 1972b). Other anterior organelles such as conoidal rings, the apical pore, the canopy and subpellicular microtubules appear to be present in both H. columbae and H. velans, although Gallucci (1974b) and Desser (1972b) interpret their micrographs differently. Differences in interpretation are particularly evident in descriptions of the polar ring and ribs in ookinetes of these 2 species. Desser (1972b) interpreted structures resembling the ribs described by Gallucci (1974b) in H. columbae as an empty subpellicular space and stated that the subpellicular microtubules in H. velans arose from

a dense ring anterior to the ribs rather than from a polar ring, as is true of most sporozoans. Additional studies of the ookinetes of H. velans or other haemoproteids transmitted by Culicoides are needed to determine whether these differences are important.

Few studies of the schizonts and sporogonic stages of avian haemoproteids have been conducted. Bradbury and Gallucci (1971, 1972) examined the fine structure of schizonts and differentiating merozoites of H. columbae. They clarified a number of inconsistencies and errors made by Aragão (1908) in his description of schizonts from lung tissue in a Rock Dove. Bradbury and Gallucci (1972) failed to find evidence of sexual dimorphism in the schizonts they examined and suggested that the differential staining noted by Aragão (1908) was probably due to differences in stage of development. Dessler (1970a) described a conspicuous sexual dimorphism in merozoites of Leucocytozoon simondi, but Bradbury and Gallucci (1971) failed to find similar differences among mature merozoites of H. columbae.

Bradbury and Gallucci (1971) also clarified the use of the term "cytomere". Aragão (1908) and later Bray (1960) used the term to refer to development of separate, uninucleate masses that eventually underwent extensive nuclear division to produce merozoites. Bradbury and Gallucci (1971) found that the schizonts of H. columbae

underwent a number of cleavages to increase the surface area available for merozoite budding. Since they did not observe the detachment of separate nucleated masses from the parent schizont, they suggested that the term "pseudocytomere" as described by Garnham (1951) should be applied to descriptions of H. columbae.

Based on their study of mature merozoites of H. columbae and their comparisons to merozoites of other haemosporidia, Bradbury and Gallucci (1972) felt that Haemoproteus and Plasmodium were more closely related than Leucocytozoon. Mature merozoites of both Plasmodium and Haemoproteus appear structurally identical, while those of Leucocytozoon differ in number of limiting membranes, overall shape, absence of cytostomes and absence of a mitochondrion - associated spherical body (Bradbury and Gallucci, 1972).

Studies of the sporogonic stages of avian haemoproteids have been limited to the sporozoites of H. columbae. Klei (1972) and Klei and DeGiusti (1973) failed to find significant differences between the structure of these sporozoites and those of other haemosporidians. The only ultrastructural study of a haemoproteid oocyst was conducted by Sterling and DeGiusti (1974) on H. metchnikovi, a parasite of turtles that is transmitted by the tabanid fly, Chrysops callidus. The small size of the oocysts they observed as well as the

formation of sporozoite buds from a single residual body closely resembled oocyst development described by light microscopy for avian haemoproteids transmitted by Culicoides (Fallis and Bennett, 1960; Kahn and Fallis, 1971).

Objectives

Haemoproteus meleagridis was first reported in a domestic turkey, Meleagris gallopavo, in Texas by Morehouse (1945). Since then, this parasite has been found in wild and domestic turkeys throughout the Nearctic range of the host and in Venezuela (Greiner and Forrester, 1980). Eve et al. (1972) and Eve et al. (1972) speculated that H. meleagridis might be a potential pathogen of wild and domestic birds. Much of the evidence for this was circumstantial, at best, and based on observations that high parasitemias seemed to coincide with periods of high mortality in wild birds.

The existence of a domesticated host that is inexpensive and available throughout the year has made H. meleagridis an attractive laboratory model for the study of avian haemoproteids. Until recently this model has not been feasible because the vectors of H. meleagridis have been unknown. In a survey of ectoparasites from

309 eastern Wild Turkeys collected in the southeastern U.S., Kellog et al. (1969) only found hippoboscids flies occasionally. Forrester (unpublished) has never recovered hippoboscids from Florida Wild Turkeys. Despite the uncommon occurrence of hippoboscids flies on Wild Turkeys, the prevalence of Haemoproteus infections is high. Forrester et al. (1974) found 69% of 85 Wild Turkeys from northern Florida and 87% of 399 Wild Turkeys from southern Florida infected with this parasite. Other workers have found prevalences ranging from 5% in Pennsylvania (Kozicky, 1948) to 80% in southern Texas (Cook, et al., 1966). The rarity of hippoboscids flies, the high prevalence of Haemoproteus infections and the fact that transmission of the parasite to caged domestic turkeys readily occurs when they are placed in a suitable habitat (Forrester, et al., 1974) suggest that ceratopogonids are the primary vectors of H. meleagridis in Florida. In confirmation of this, Atkinson et al. (1983) recently demonstrated that at least 3 species of Florida Culicoides could support complete development of the sporogonic stages of H. meleagridis.

This study was undertaken to

(1) determine the vectors and investigate the seasonal transmission and epizootiology of H. meleagridis in Florida,

(2) study the pathogenicity of H. meleagridis in domestic turkeys with controlled experimental infections,

(3) examine the host specificity of the parasite and

(4) study the fine structure of the H. meleagridis in the vertebrate and invertebrate hosts.

MATERIALS AND METHODS

Epizootiology

Sentinel Study

Between 9 May, 1982, and 15 July, 1984, groups of 3, 2-week-old, Broad-breasted white domestic turkey poults were exposed for 2-week periods in sentinel cages placed at 2 sites at Paynes Prairie State Preserve 2 km SSE of Gainesville, Florida. One site (A) was located in a mixed, deciduous forest and had 2 sentinel cages, 1 at ground level and a second suspended from a rope hoist 7 m above the first. The second site (B) was approximately 1 km from the first and was located in an ecotone between the forest and an open field. At the second site, a single cage was placed on the ground in a small grove of oak trees, approximately 30 m from the edge of the main forest. All 3 sentinel cages were screened with 2 layers of 1.3 cm hardware cloth to allow entry of vectors and to restrict entry of large predators. Sentinel turkeys were fed a high protein, commercial, unmedicated game bird chow ad libitum and watered regularly throughout each 2-week sentinel period. At the end of the 2-week sentinel period, the birds were moved in a vector-proof

cage to Culicoides-proof turkey rooms and held for 4 weeks to allow any infections acquired in the field to become patent. They were replaced in the field on the same day with unexposed, 2-week-old poults. The sentinel birds were bled from a leg vein, 3 times a week for 4 weeks, following their exposure in the field. Blood smears were fixed with absolute methanol and stained with 10% Giemsa, pH 7.2. Infections were diagnosed by scanning approximately 10,000 red blood cells at 1000X. All turkeys used in this study were obtained as day-old poults from Thaxton's Turkeys (P.O. Box 127, Watkinsville, Georgia).

Vectors

Once every 2 weeks a Bennett trap (Bennett, 1960) and a New Jersey light trap were operated at each site within 50 m of the sentinel cages. The Bennett trap was operated in the middle level of the forest canopy where ornithophilic species of Culicoides are most active (Tanner and Turner, 1974) from approximately 1 hour before sunset to 1 hour after sunset. A turkey was placed into an 0.3 cubic meter welded wire cage made from 1.3 cm mesh and hoisted on an 0.6 square meter plywood board into the canopy by means of lightweight nylon rope and small pulleys. Following an exposure of 10 - 20 minutes, the bird was lowered quickly to the ground and covered with an 0.6 cubic meter wooden frame screened with fine nylon

mesh (28 mesh per cm). Two domestic turkeys that were the same age and size were hoisted alternately into the canopy. While 1 bird was exposed, the second was left undisturbed under the screened wooden frame for 10 minutes to allow specimens of Culicoides to complete their blood meals. Engorged and unengorged individuals of each species of Culicoides were then aspirated through a sleeve in the top of the outer screened frame as they rested on its interior.

Specimens of Culicoides aspirated during each run of the Bennett trap were placed into half-pint cardboard cartons with screened tops and supplied with a cotton pad moistened with 5% (w/v) sucrose. The beginning and ending times for each run of the Bennett trap, temperature, wind velocity estimated by the Beaufort scale (Oliver, 1973) and overall weather conditions were recorded. Since early attempts at Bennett trapping were unsuccessful in rain and when wind velocity exceeded Beaufort 3 (4-7 miles/hr.), all trapping throughout the course of the study was done on calm evenings when rainfall was not imminent. Between 1 and 9 days after capture, the specimens of Culicoides were identified (Blanton and Wirth, 1979) and classified according to parity by the method of Dyce (1969). Bennett trap catches of individuals of each species were expressed as the \log_{10} of the number captured, plus 1. When sampling was done on more than

1 night during a sentinel period, the geometric mean of all samples was calculated (Bidlingmeyer, 1969).

A standard New Jersey light trap (Hausherr's Machine Works, Old Freehold Rd., Toms River, NJ) equipped with a 40 watt incandescent bulb, an automatic timer and a delivery cone made from 40-mesh brass was operated at each of the 2 sites 1 night approximately every 2 weeks. Two to 3 kg of dry ice were placed in an insulated paper envelope and hung next to the top of the trap to act as a carbon dioxide attractant. The trap was operated in the middle levels of the canopy at the same spot where the Bennett trap had been placed. Sampling with the New Jersey trap usually followed or preceded operation of the Bennett trap by 1 day. The trap was powered by a portable 500 watt gasoline generator and was started 30 minutes to 1 hour before sunset and run until dawn. Insects were collected into a 1 pint mason jar containing 10% buffered formalin and a small amount of detergent (Alconox, Fisher Scientific). Aliquots of each sample were poured into a white enamel pan and diluted with water. Specimens of Culicoides were picked from each aliquot with a Pasteur pipette, identified, grouped by sex and parity and counted. New Jersey light trap data for each species were expressed as the \log_{10} of the number captured per trap night, plus 1. The geometric mean was

calculated when more than 1 sample was taken during a sentinel period (Bidlingmeyer, 1969).

Between December, 1982, and November, 1984, collecting trips were made approximately once a month to Lykes Fisheating Creek Wildlife Management Area at Palmdale, Florida, 310 km SSE of Paynes Prairie. This area has one of the most dense Wild Turkey populations in the state (Powell, 1967; L.E. Williams, pers. comm.). Earlier sentinel work by Forrester (unpublished) had shown that H. meleagridis was transmitted year-round in this area.

A study site was selected in a live oak hammock, surrounded by cypress, at the edge of a creek swamp, 5 km SSE of Palmdale. During each collecting trip, a Bennett trap was operated in the middle level of the canopy for 1-3 consecutive evenings and occasionally at night and during the day. The New Jersey light trap, supplemented with dry ice, was operated 1 night/trip in the middle level of the canopy at a second hoist, 30 meters from the first. Three to 5, 2-week-old domestic poults were also exposed at the study site for the duration of each trip. They were housed, as described earlier, 1 meter above the ground in a sentinel cage. The birds were transported to and from Gainesville in a screened, vector-proof cage and bled as described earlier to diagnose infections.

Meteorological data for the study sites at Paynes Prairie and Fisheating Creek were obtained from the nearest weather stations, operated on a continuous basis (Climatological Data: Florida, 1982, 1983, 1984). In northern Florida, data were obtained from the U.S. weather station operated at the Gainesville airport, located approximately 8 km N of the study areas at Paynes Prairie. In southern Florida, data were obtained from the U.S. weather station at LaBelle, approximately 24 km SW of the study site at Fisheating Creek.

Activity Cycles

Capture times were determined for specimens of C. edeni, C. hinmani, C. arboricola and C. knowltoni obtained in Bennett traps at Paynes Prairie and Fisheating Creek. Capture times for individual specimens of Culicoides were calculated as the midpoint of the Bennett trap run when they were collected. The data were plotted by species, site, year and quarter (January-March, April-June, July-September and October-December) as the number of minutes before or after nautical sunset on the date of capture. Sunset times were calculated from standard tables (Nautical Almanac, Nautical Almanac Office, U.S. Naval Observatory) and adjusted for the proper latitude and longitude of each study site. Scatter plots for individuals of each species were very similar when examined

by site, year and quarter. To determine whether capture data from each site, year and quarter could be combined by species and analyzed as a single data set, the mean capture time, standard deviation and sample size was computed for each species by site, year and quarter. Because some species were not active during all quarters, and a number of empty "cells" were present when mean capture times were examined by site, year and quarter, it was assumed that year had a minimal effect on variability in the data. Data for each species from each of the 3 years of the study were combined by site and quarter. An analysis of variance using the Statistical Analysis System, general linear models procedure was used to test for significant interaction effects between species*site, species*quarter, site*quarter and species*site*quarter (SAS User's Guide: Statistics, 1982). An alpha level of 0.05 was considered significant. Species*site and species*quarter interactions were significant ($p < 0.0001$). The analysis was repeated without the Fisheating Creek data to determine whether data from the 2 sites at Paynes Prairie could be combined by quarter. Species*quarter interactions were significant ($p < 0.0001$). The final analysis was done by site (Site A and B combined) and quarter with a one-way analysis of variance using the SAS general linear models procedure (SAS User's Guide: Statistics, 1982). Significant species

effects were tested with a Duncan's Multiple Range Test. When fewer than 3 species were compared, a T-test was used (SAS User's Guide: Basics, 1982).

A pair of New Jersey light traps, modified as described earlier and baited with a paper envelope containing 2-3 kg of dry ice, was operated continuously for 24-36 hours at Fisheating Creek during the March, April and May, 1983, collecting trips. One trap was suspended 1 meter above the ground and the second, 7 meters above it, in the middle level of the canopy. Both traps were operated without light bulbs to minimize diurnal and nocturnal differences in their attractiveness to biting arthropods. Sample bottles from each trap were changed every 2 hours between 1700 and 0900 hours and every 4 hours between 0900 and 1700 hours. Dry ice was replenished every 4 hours. Data were plotted by as the average number of individuals of each species of Culicoides captured per hour of trap time for each sampling period.

Experimental Infections

To test the ability of individuals of various species of Culicoides to support development of H. meleagridis, infected sentinel turkeys were exposed in the Bennett trap. Engorged flies were dissected at daily intervals up to 9 days after a blood meal was taken in 0.85 % (w/v)

saline or Aedes aegypti ringers (Hayes, 1953). The flies were removed from their cartons with an aspirator and blown into a small petri dish containing saline and a drop of Triton 100 X (Fisher Scientific). The specimens of Culicoides were then transferred to a glass microscope slide and identified by wing pattern under a dissecting scope (Blanton and Wirth, 1979). The salivary glands and midgut were carefully removed with fine dissecting pins (minuten nadeln) in a drop of saline, covered with a coverslip and examined at 400X for oocysts and sporozoites with Normarski contrast interference microscopy. Questionable identifications of individual specimens of Culicoides were confirmed after dissected flies had been cleared overnight in liquid phenol and mounted on a microscope slide in a drop of 50% liquid phenol and 50% Canada balsam. Engorged midguts were smeared on glass slide, air dried, fixed in absolute methanol and stained with Giemsa as described earlier. Measurements of ookinetes were made from camera lucida drawings and adjusted to scale with a slide micrometer.

Domestic poultts were infected by drawing salivary glands containing sporozoites into the needle of a tuberculin syringe and injecting them intraperitoneally (IP) or intravenously (IV) into uninfected poultts. Whole flies were also ground in 0.85% saline, Aedes aegypti ringers (Hayes, 1953) or RPMI tissue culture medium

containing 10% turkey serum in a glass tissue grinder in wet ice for several minutes. The resulting slurry was then inoculated as described into uninfected poults. Estimates of the total number of sporozoites inoculated were made with a hemocytometer. Permanent sporozoite preparations were made by mixing the slurry 1:10 with turkey serum. A drop was smeared on a glass slide, air dried and fixed and stained as above. Measurements of stained sporozoites were made as above.

Estimation of Prevalence

During operation of the Bennett trap, only 50-70% of captured specimens of Culicoides normally took a blood meal from the bait turkey. Unengorged specimens of Culicoides were identified by wing pattern, grouped by species and ground in lots of 10-20 in Aedes aegypti ringers or RPMI tissue culture media, as described earlier. The slurry from each pool was inoculated intraperitoneally into separate 1- to 2-week old turkey poults. These birds were bled as described earlier to diagnose any infections. Minimum yearly prevalence of H. meleagridis in pools of naturally infected C. edeni was calculated for Paynes Prairie and Fisheating Creek as the total number of positive pools / total number of specimens of C. edeni.

PathogenicityExperiment 1 - Pathology

Thirty-six, 1-day-old, female, broad-breasted white turkey poults were obtained in October, 1984. They were housed together in a brooder in a vector-proof room for 7 days, then banded with metal wing tags and randomly assigned to 3 experimental groups. Birds in the first group were inoculated IP with separate pools of 5 C. edeni that had taken blood meals from 4 domestic poults infected with H. meleagridis. The specimens of Culicoides were captured in October, 1984, at Fisheating Creek with a Bennett trap, held for 8-9 days at room temperature to allow development of sporozoites and then ground by hand in a glass tissue grinder for several minutes in an ice bath. The insects were triturated in RPMI tissue culture medium containing 10% turkey serum. The 4 domestic poults used to infect the wild-captured specimens of C. edeni had acquired their infections from a previous exposure at Fisheating Creek. Capture of specimens of C. edeni occurred during 2 evenings on days 9 and 10 of patency when most gametocytes were fully mature. All 4 birds had similar parasitemias. Engorged C. edeni from both trap nights were assigned randomly to pools used to infect the experimental poults. Sporozoites from 1 pool of specimens of C. edeni were counted with a hemocytometer

to determine the approximate dosage. Each of the 12 birds was inoculated with 0.5 cc of slurry containing approximately 4,400 sporozoites.

Birds in the second experimental group were inoculated IP with separate pools of 35 specimens of C. edeni, ground and quantified as above. Each bird was inoculated with 0.5 cc of slurry containing approximately 57,500 sporozoites. Birds in the control group were inoculated IP with 0.5 cc of RPMI tissue culture fluid containing 10% turkey serum.

Following inoculation the birds were housed in groups of 3 in 12 battery cages in a vector-proof room. Each compartment held 1 bird from each experimental group. Birds were assigned to the compartments with a random number table. The poults were fed and watered as described earlier.

Twenty-four hours prior to their inoculation (Week 0), and once a week for 8 weeks following infection, each bird was weighed and the tarsometatarsal length of the right leg was measured with calipers. Two heparinized capillary tubes were filled with blood from a wing vein. Blood was immediately drawn from 1 capillary tube into 2, 20 ul pipettes. The contents of each pipette was immediately dispensed into each of 2 separate test tubes containing 5 mls of cyanomethemaglobin reagent (1:251 Cyanomethemaglobin Test Kit, Fisher Scientific). The

tubes were mixed with a vortex mixer and allowed to stand for several hours. Absorbence was measured spectrophotometrically at 540 nm. Hemoglobin concentration for each paired sample was determined with a standard measured at the same time (Cyanomethemoglobin Standard, Fisher Scientific). Average values for each paired sample were used in the statistical analysis.

The second hematocrit tube was spun for 5 minutes in a microhematocrit centrifuge. The packed cell volume (PCV) was measured and a drop of plasma was placed in a refractometer to determine the plasma protein concentration.

Blood smears were prepared from all birds 3 times per week as described earlier. Parasitemias were determined by counting the number of gametocytes per 10,000 red blood cells. The number of red cells in each of 5 oil immersion fields behind the leading "tongue" of the smear were counted. The average number of red cells per field was determined and the number of fields needed to scan 10,000 red cells was calculated.

At 4 weeks post-infection and again at the end of the experiment, fecal samples were collected from each compartment. Flotations were performed on the samples with Sheather's sugar solution to detect coccidian oocysts. At 4 weeks post-infection cloacal swabs were prepared from 3-5 randomly selected birds in each

experimental group, incubated overnight in selenite enrichment media and plated on MacConkey's Agar. Bacterial colonies morphologically similar to Salmonella spp. were identified by biochemical reaction with Micro ID test kits (Mallincrock Industries). Salmonella spp. isolates were sent to the National Veterinary Diagnostic Laboratory at Ames, Iowa for further typing. At the termination of the experiment, cloacal swabs were made from all surviving birds and screened for Salmonella spp. as above.

At 8 weeks post-infection, all surviving birds were killed by electrocution and necropsied. Wet weights of heart, liver and spleen (expressed as percent of total body weight at necropsy) were measured. Representative pieces of pectoral muscle, liver, spleen, heart, lung, brain, proventriculus, gizzard, duodenum, pancreas, ileum, jejunum, cecum, kidney and bone marrow taken from the femur were fixed in 10% buffered formalin. The 3 lightest birds from each group were selected and all representative tissues from each were dehydrated in ethanol or isopropyl alcohol, cleared in toluene for 2 hours, embedded in paraplast, sectioned at 5 μ m and stained with hematoxylin and eosin. Representative tissues from birds that died prior to the end of the experiment were fixed in 10% buffered formalin and Carnoy's fixative, dehydrated, cleared, sectioned and stained as above. Selected serial sections of skeletal muscle were stained for calcium with

von Kossa's stain (Humason, 1979). Pectoral muscle from 2 of these birds was fixed and processed for electron microscopy as described later.

Data on weight, tarsometatarsal length, hematocrit, plasma protein concentration and hemoglobin were analyzed with the SAS general linear models procedure as a split-plot design with subjects as main plot units and subjects at a particular time as a subplot unit (Freund and Littell, 1983). Treatment, subject(treatment), week and treatment*week were tested for each variable using the Type III sum of squares. A p value of 0.05 or smaller was considered significant. When treatment*weeks interactions were significant, further comparisons were made by treatment and by week with Duncan's Multiple Range Test. A comparison of organ weights at necropsy was done with a one-way analysis of variance using the SAS general linear models procedure (SAS User's Guide: Statistics, 1982).

Experiment 2 - Exoerythrocytic Development

A second series of experimental infections was conducted to study the development of the early exoerythrocytic stages and their associated host response. Four domestic turkeys, infected as sentinel birds at Fisheating Creek, were exposed in Bennett traps at Fisheating Creek in May, 1985, on days 8-10 of patency.

Engorged specimens of C. edeni and C. hinmani were collected and held as described earlier for 7 to 9 days. They were randomly assigned to pools of 48 specimens of C. edeni and 33 specimens of C. hinmani, triturated as described earlier and inoculated IP into 6, 5-day-old, female broad-breasted white turkey poults. One pool was ground and quantified with a hemocytometer to estimate total sporozoite dose. Each bird was inoculated with 1.0 cc of slurry containing approximately 169,000 sporozoites. Six control birds of the same sex and age were inoculated intraperitoneally with 1.0 cc of the carrier.

On days 3, 5, 8, 11, 14 and 17 post-infection, 1 inoculated poult and 1 control bird were killed by decapitation. Representative pieces of pectoral muscle, liver, spleen, lung, heart, brain, kidney, bone marrow, duodenum, pancreas and cecum were fixed in 10% buffered formalin and Carnoy's fixative. Tissue fixed in Carnoy's was dehydrated in absolute ethanol, cleared overnight in amyl acetate and embedded in paraplast. Sections were cut at 4 μ m and stained with hematoxylin and eosin or Giemsa-colophonium (Bray and Garnham, 1962).

Host SpecificityInfection of Hosts

In June and July, 1984, domestic turkeys infected with H. meleagridis were used as bait birds in Bennett traps operated at Fisheating Creek. Engorged specimens of C. edeni and C. hinmani were collected, held at 25°C for 7 days to allow development of sporozoites, pooled and ground in Aedes aegypti Ringers (Hayes, 1953). The slurry from the first pool of 40 specimens of C. edeni and 32 specimens of C. hinmani, collected in June, was divided equally among 2, 2-day-old Chukar Partridges, Alectoris chukar, 2, 2-day-old Guinea fowl, Numida meleagris, 2, 7-day-old Ring-Necked Pheasants, Phasianus colchicus, and 2, 7-day-old broad-breasted white turkey poults. The Chuckars, Guinea fowl and Ring-necked Pheasants were obtained from Morris Hatchery (Miami, Florida). Each bird was inoculated IP with 0.15 cc of slurry. Sporozoite counts of the slurry with a hemocytometer revealed that each bird received approximately 375 sporozoites. Pairs of uninfected Chuckars, Guinea fowl, Ring-necked Pheasants and turkeys were kept as negative controls. All birds were housed by species in separate battery cages in a vector-proof room and fed and watered as described earlier. Smears were prepared from blood obtained from leg veins of all birds, 3 times a week,

for 4 weeks following inoculation. They were fixed and stained as described earlier.

The slurry from a second pool of 37 engorged specimens of C. edeni and 84 engorged specimens of C. hinmani, collected in July, 1984, was divided equally among 2, 3-day-old Northern Bobwhites, Colinus virginianus, 2, 7-day-old Rhode Island red chickens, Gallus gallus, and 2, 5-day-old broad-breasted white turkeys. The chickens were obtained from a local hatchery and the quail were obtained from the Department of Poultry Science, University of Florida. Each bird was inoculated IP with 0.1 cc of slurry containing approximately 5,000 sporozoites. A pair of uninfected birds of each species was kept as negative controls. All birds were maintained and bled as described earlier. Tissues from birds that died prior to the end of both experiments were fixed in 10% buffered formalin and embedded, sectioned and stained as described earlier.

Morphometric Analysis

Parasitemias were quantified as the number of parasites per 10,000 red blood cells as described earlier. Morphometric parameters were determined from a maximum of 15 mature, 7- to 9-day-old microgametocytes and 15 mature, 7- to 9-day-old macrogametocytes from each host. Measurements were made by the methods of Bennett and Campbell (1972) as modified by Forrester et al. (1977).

Fifteen uninfected red cells were also measured from each host. All measurements were made from camera lucida drawings of infected and uninfected erythrocytes that were adjusted to the proper scale with a slide micrometer.

A discriminant analysis was performed on measurements of microgametocytes, macrogametocytes and infected host cells from each species susceptible to H. meleagridis to determine whether parasite and host cell morphology differed in each host. Since uninfected red cells from each host species differed in size and could have a limiting effect on parasite size, measurements of parasites were expressed as a percentage of the average area of uninfected red cells from the same host. To standardize morphological changes in host cells infected with gametocytes, measurements from infected host cells were expressed as a fraction of the corresponding average measurement of uninfected host cells of the same species (e.g. infected host cell area/average host cell area). Before analysis, all variables were tested for normality with the Shapiro-Wilk statistic (SAS User's Guide: Basics, 1982). Since approximately 1/4 of the variables were non-normal, a nonparametric, nearest neighbor analysis ($k=3$) was performed (SAS User's Guide: Statistics, 1982).

Adjusted measurements of macrogametocyte length and width, nucleus length and width, gametocyte area, nucleus area and number of pigment granules were used as predictor variables to generate a discriminant function based on

Mahalanobis distances (Kachigan, 1982). A similar function was derived for microgametocytes using adjusted measurements of gametocyte length and width, gametocyte area and number of pigment granules. Measurements of microgametocyte nuclei were not included because they were diffuse and poorly defined in Giemsa-stained blood smears (Greiner and Forrester, 1980). Two additional discriminant functions were derived for red blood cells containing macrogametocytes and for red blood cells containing microgametocytes. Adjusted measurements of host cell length and width, host nucleus length and width, host cell area, host nucleus area and nuclear displacement ratio were used to derive the functions. The efficacy of each of the 4 discriminant functions was tested using adjusted measurements of a fresh sample of 3 or 4 gametocytes and host cells from each infected host species.

Fine Structure

All tissue processed for electron microscopy was fixed in 3% (v/v) glutaraldehyde in 0.1% (w/v) sodium cacodylate buffer with 4% (w/v) sucrose, pH 7.2. Tissue was post-fixed in 1% (w/v) osmium tetroxide in the same buffer, stained en bloc with 2% uranyl acetate in 75% ethanol, dehydrated through a graded ethanol series,

cleared in acetone and embedded in Spurr's resin. Ultrathin sections were cut on glass knives, stained with 5% (w/v) aqueous uranyl acetate and 2% (w/v) Reynold's lead citrate and examined with a Hitachi HU-11E electron microscope.

Mature gametocytes were fixed by drawing blood from the wing vein of a turkey infected with H. meleagridis into a syringe containing the primary fixative. The resulting clots were diced in the primary fixative, fixed for 1 hour at room temperature, washed with 3, 10-minute changes of buffer, post-fixed with osmium for 1 hour at room temperature, washed with 3 more 10-minute changes of buffer and dehydrated and embedded as described above. Several drops of fresh blood from the same turkey were placed on a glass slide in a humidity chamber to allow the gametocytes to exflagellate. Two minutes and 3 minutes after the drops were made, the clots were flooded with primary fixative and processed as above.

Specimens of C. edeni that had engorged on a turkey with a heavy H. meleagridis infection, 3 and 6 days earlier, were dissected in a drop of Aedes aegypti Ringer's. The midguts were carefully removed, flooded with a drop of primary fixative and processed through the first series of washes. To facilitate handling and to prevent loss during subsequent steps, the midguts were embedded in warm 2% agar made with 0.1 M sodium cacodylate

buffer with 4% sucrose, pH 7.2. After they were cut into small blocks, processing continued as above.

Pectoral muscle containing megaloschizonts was diced in primary fixative and processed as described above.

RESULTS

Epizootiology

Vectors

Paynes Prairie. During 96 nights of trapping between May, 1982, and July, 1984, 34,342 specimens of Culicoides belonging to 27 species were captured in New Jersey light traps at Sites A and B (Table 2). Culicoides insignis was the most common species taken at Paynes Prairie. However, 79% of the total catch of 18,996 individuals was captured in a single night at Site B in November, 1982. Specimens of 9 additional species, i.e. C. edeni (10.2%), C. stellifer (8.7%), C. arboricola (8.1%), C. crepuscularis (4.1%), C. spinosus (3.2%), C. scanloni (2.0%), C. nanus (2.0%), C. debilipalpus (1.5%) and C. niger (1.4%), made up approximately 40% of the remaining catch. Specimens of the other 17 species were captured infrequently or in low numbers.

Of the specimens of 27 species of Culicoides captured in New Jersey light traps, only representatives of 12 species, totaling 1,428 engorged individuals, were taken in

turkey-baited Bennett traps at both sites. The traps were operated on 115 different evenings for a total of 211 hours (Table 3). Culicoides edeni (48.0%) and C. hinmani (26.2%) were the most common species and, together, made up 74.2% of the combined catch from each site. Specimens of C. scanloni (7.9%), C. arboricola (7.6%) and C. nanus (5.1%) composed 20.6% of the total. Representatives of the remaining 7 species made up 5.1% of the total Bennett trap catch.

Fisheating Creek. During 28 nights of trapping between December, 1982, and November, 1984, 17,857 specimens of Culicoides belonging to 13 species were captured in New Jersey light traps at Fisheating Creek (Table 4). Culicoides insignis (44.5%) and C. edeni (40.1%) were the most common species and composed 85.2% of the catch. Culicoides knowltoni (7.6%) and C. stellifer (4.2%) were less common and made up 11.8% of the catch. Representatives of the remaining 9 species were captured infrequently or in low numbers and composed only 3% of the total catch.

During the same period, 2,561 engorged individuals of 5 species of Culicoides were captured in turkey-baited Bennett traps operated on 47 different evenings for a total of 108 hours (Table 5). Approximately 98% of the total catch was composed of specimens of C. edeni (79.6%) and C.

hinmani (18.6%). The remaining catch was made up of specimens of C. knowltoni (1.4%), C. arboricola (0.5%) and C. baueri (0.04%).

Experimental Infections

Sporogonic development. Engorged individuals of 10 species of Culicoides were collected from Bennett traps baited with turkeys infected with H. meleagridis. Fresh preparations and Giemsa-stained smears of engorged midguts; dissected from specimens of C. edeni within 24 hours after a blood meal was taken, had numerous ookinetes. In fresh preparations, ookinetes had a refractile "knob" or "point" at one end and a mass of golden-brown pigment at the other (Figure 1). Fifteen Giemsa-stained ookinetes measured 16.5-21.1 μm in length (Mean = 18.95, SD = 1.4) and 2.5-3.75 μm in width (Mean = 2.98, SD = 0.458). The nucleus was oval to round and measured 2.0-3.25 μm in length (Mean = 2.48, SD = 0.417) and 1.5-2.25 μm in width (Mean = 1.98, SD = 0.32). In well stained preparations, 1, and occasionally 2, empty vacuoles, slightly smaller than the ookinete nucleus, were located anterior and/or posterior to it.

Dissections of specimens of Culicoides, from 2 to 7 days after they had taken blood meals from infected turkeys, revealed both viable and degenerating oocysts

on the outer wall of the midguts (Figures 2, 3, 4). Representatives of 5 species of Culicoides, i.e. C. edeni, C. arboricola, C. haematopotus, C. hinmani and C. knowltoni, were able to support complete development of H. meleagridis and had mature oocysts, packed with sporozoites, and salivary glands with numerous slender sporozoites by 6 to 7 days after they had taken blood meals (Table 6) (Figures 4, 6). Culicoides edeni was the most susceptible species. Almost 2/3 of the engorged specimens of C. edeni developed salivary gland infections (Table 6). Four oocysts from C. edeni were subspherical and measured 14-16.5 μm in length (Mean = 15.4, SD = 1.11) and 12-16.5 μm in width (Mean = 14.1, SD = 1.84). Oocysts contained from 50-100 elongate sporozoites that were aligned parallel to one another. A small, eccentric residual body composed, in part, of golden-brown pigment granules was present in each oocyst (Figure 4). Salivary gland sporozoites were within secretory cells of the single major lobe that composed each of the 2 glands (Figure 5). In fresh preparations, they often flexed and twisted within the salivary gland. Fifteen Giemsa-stained sporozoites from 1 specimen of C. edeni measured 9.25-12.5 μm in length (Mean = 11.1, SD = 0.8) and 0.5-1.0 μm in width (Mean = 0.69, SD = 0.17). The nucleus was located approximately 1/3 of the total length from one

end and measured 1.0-2.0 μ m in length (Mean = 1.69, SD = 0.33) and 0.5-1.0 μ m in width (Mean = 0.74, SD = 0.19).

Specimens of 4 species of Culicoides, i.e. C. paraensis, C. nanus, C. scanloni and C. baueri, were capable of supporting partial development of H. meleagridis and had degenerating oocysts on their outer midguts by 4 to 7 days after taking a blood meal (Table 6). Degenerating oocysts were smaller than mature, 7-day-old oocysts and contained large refractile granules (Figure 3). No development was observed in 3 specimens of C. crepuscularis (Table 6).

Experimental transmission. Salivary gland sporozoites from specimens of C. edeni, C. hinmani, and C. arboricola infected 12 of 20, 1 of 6 and 1 of 2 domestic turkeys, respectively, when inoculated IP or IV. One pool of 5 specimens of C. knowltoni, ground in Aedes aegypti Ringer's, infected a domestic poult. A second pool of 14 specimens of C. knowltoni was negative, when inoculated into another poult. Salivary gland sporozoites from a single specimen of C. haematopotus did not infect a domestic poult.

The prepatent period of all successful infections ranged from 17-18 days, with peak red cell invasion occurring by 18-19 days. The rate of growth and morphology of immature and mature gametocytes was consistent with

descriptions of neotypes of H. meleagridis (Greiner and Forrester, 1980).

Activity Cycles

Bennett trap catches. Of the 5 species of Culicoides capable of supporting development of H. meleagridis, individuals of only 4 species, C. edeni, C. hinmani, C. arboricola and C. knowltoni, were captured in sufficient numbers to permit analysis of their times of capture. A scatter plot of capture times for specimens of C. hinmani from 2.5 hours before sunset to 2.5 hours after sunset was unimodal with a peak at 41.6 minutes before sundown (Figure 7). Scatter plots of capture times for specimens of C. edeni, C. arboricola and C. knowltoni were also unimodal, but average peaks were 10.3, 19.2 and 28.3 minutes after sundown, respectively (Figures 8, 9, 10).

Comparisons of mean capture times among individuals of the 4 species revealed the same trends at each site (Paynes Prairie and Fisheating Creek) and during each quarter (January - March, April - June, July - September, October - December) (Table 7). Specimens of C. hinmani had a peak in biting activity from 24 to 59 minutes before sunset that was significantly earlier than individuals of the other species. Significant differences among mean capture times

for specimens of C. edeni, C. arboricola and C. knowltoni were less clear and varied from quarter to quarter. At Fisheating Creek, mean capture times for specimens of C. arboricola and C. knowltoni were not significantly different during any quarter. However, the peak biting activity for specimens of C. edeni was significantly earlier than specimens of C. arboricola during quarters 2 and 4, but not during quarters 1 and 3. At Paynes Prairie, specimens of C. edeni had a peak capture time that was significantly earlier than specimens of C. arboricola during quarters 3 and 4, but not during quarter 1. During quarter 2, specimens of C. edeni had a peak in activity that was significantly later than specimens of C. arboricola (Table 7).

In spite of these differences, the same trend was evident at each site. Specimens of C. hinmani reached a peak in biting activity before sunset. They were followed from 0 to 19 minutes after sunset by specimens of C. edeni. Specimens of C. arboricola were most active from 8 to 55 minutes after sunset. Specimens of C. knowltoni were the last to become active between 26 and 51 minutes after sunset. When mean capture times for individuals of each species are compared between Paynes Prairie and Fisheating Creek, differences are usually within 1 standard deviation of each other.

New Jersey trap catches. Culicoides edeni and Culicoides hinmani were the most common species collected in the CO₂-baited New Jersey suction traps. During the March, April and May, 1983, collecting trips to Fisheating Creek, specimens of C. edeni had a peak in activity during the 2-hour sampling period that included sunset (Figure 11). Individuals of this species were active at low levels during the night in March and April. During all 3 collecting trips, activity increased following sunrise and continued throughout the day at levels lower than the evening peak. During the day, specimens of C. edeni were often observed crawling on the head and on the back feathers of sentinel turkeys exposed on the ground. Most individuals of C. edeni were captured in the suction trap that was operated in the canopy (Figure 11).

During the April and May collecting trips, specimens of C. hinmani had peaks in activity during the 2-hour sampling period that included sunset and during the early morning hours following sunrise (Figure 12). Activity during the April trip continued into the early afternoon. Similar diurnal activity did not occur during the March collecting trip. All specimens of C. hinmani were captured in the canopy trap. This species was never captured in Bennett traps operated on the ground.

Sentinel Study

Paynes Prairie. Between May, 1982, and July, 1984, 30 of 327 (9.2%) sentinel poults at Site A and 32 of 140 (22.9%) sentinel poults at Site B became infected with H. meleagridis. At Site A, 6 of 166 (3.6%) exposed on the ground vs. 24 of 161 (14.9%) exposed in the canopy developed patent infections. A 2 by 2, Chi Square test of the independence of exposure height and transmission was highly significant ($p < 0.01$).

During 1982, transmission of H. meleagridis began in mid-August at Site B and mid-September at Site A (Figures 13, 14), peaked from mid-October to mid-December during periods of above average temperatures for northern Florida (Figure 15) and tapered off at the end of December at Site A and in mid-January at Site B, with the onset of cooler winter weather in January, 1983 (Figures 15, 17). As average monthly temperatures reached and exceeded 60° F (Figure 17), transmission began again in mid-April, 1983, at Site B and early May, 1983, at Site A and continued at both locations throughout the summer and fall until the onset of cooler, winter weather in mid-December, 1983 (Figures 15, 17). Deviations from the average monthly rainfall at Paynes Prairie were minor throughout most of the study. Rainfall was above average during March, April, June and

September, 1983, and below average during July and August, 1983, and July, 1984 (Figure 16).

Two peaks in transmission occurred in 1983 at each site. A small peak was evident between July and August and again between November and December at Site A. Peaks at Site B occurred 1 to 2 months earlier between May and June and between October and November. In 1984, transmission at both sites began in mid-April and continued until the end of the study in July.

Fisheating Creek. Between February, 1983, and November, 1984, 52 of 66 (78.8%) sentinel turkeys exposed during collecting trips to Fisheating Creek became infected with H. meleagridis. Exposures as short as 24 hours in November and December, 1983, and January, March and June, 1984, were sufficient to infect 100% of the 3 or 4 sentinel turkeys that were taken during each trip.

Between June, 1983, and September, 1984, 50 - 100% of the sentinel birds exposed during each trip became infected with H. meleagridis (Figure 18). Transmission did not occur in February, March and April, 1983, during periods of abnormally cool and wet weather (Figures 19, 20). Transmission was not detected in November, 1984, when monthly precipitation was above average and mean temperatures were slightly below normal (Figures 19, 20).

Throughout the study at Fisheating Creek, average monthly temperatures never fell below 60o F.

Transmission and Vector Abundance

Culicoides edeni, C. hinmani, C. arboricola and C. knowltoni were the only species captured in sufficient numbers in Bennett traps, to be implicated as potential vectors at Paynes Prairie and Fisheating Creek. Because representatives of all 4 species had distinctive peaks in activity at sunset, biting activity was plotted for each as the log₁₀ of the number captured during the peak Bennett trap run for a sampling night, plus 1.

Paynes Prairie. During the 2-year study at Paynes Prairie, biting collections and light trap collections for each species were positively correlated (Figures 13, 14). Specimens of C. edeni were active at variable levels throughout the year. Bennett trap and light trap catches were usually lowest during the cooler, winter months between January and March (Figures 13, 14, 18). Transmission of H. meleagridis to sentinel birds began soon after the biting activity and abundance of C. edeni increased in late April, 1983 and 1984, with the onset of warmer spring weather. Peaks in the transmission of H. meleagridis to sentinel turkeys corresponded to minor peaks

in the biting activity of C. edeni in July and December, 1983, at Site A (Figure 13) and in December, 1982, and May, 1983, at Site B (Figure 14). Transmission did not occur between May and September, 1982, at either site, in spite of relatively large, but variable, catches of C. edeni (Figures 13, 14). Similar anomalies between the transmission of H. meleagridis to sentinel birds and the abundance and biting activity of C. edeni occurred in October, 1982, and October, 1983, at site B (Figure 14) when catches of C. edeni were low, in spite of high rates of transmission.

Culicoides hinmani was active only during the warmer months of the year, between May and October. Both biting activity and abundance were essentially unimodal in distribution, with variable peaks between May and October during periods of active transmission of H. meleagridis to sentinel birds. Culicoides hinmani was absent during periods of high transmission of H. meleagridis in December, 1982, and December, 1983 (Figures 13, 14).

Culicoides arboricola was absent at both sites in 1983 and 1984 during the cooler winter months of January, February and March. Peaks in biting activity occurred in September, 1982, April and September, 1983, and March, 1984, at each site. Light trap collections during the same period at Site B had a similar, bimodal

distribution. A bimodal distribution was less evident in light trap collections from Site A (Figures 13, 14). Biting activity of C. arboricola was very low or absent during periods of high transmission of H. meleagridis between November and December, 1982, and between October and December, 1983.

Fisheating Creek. Bennett trap and light trap collections of C. edeni, C. hinmani and C. arboricola from Fisheating Creek had seasonal patterns that were similar to catches from Paynes Prairie. Culicoides edeni remained active throughout the year, with fewer numbers and lower biting activity from December, 1982, through March, 1983, and January and November, 1984, during cooler winter weather (Figure 18). Haemoproteus meleagridis was transmitted at high levels to exposed sentinel birds between May, 1983, and October, 1984.

Culicoides hinmani and C. arboricola were absent or present in low numbers from December, 1982 - March, 1983, and from December, 1983, and March, 1984, when average monthly temperatures were lowest (Figures 17, 18). Light trap and Bennett trap collections of C. hinmani were bimodal in distribution in 1983 with peaks in May and September. The distribution was more unimodal in 1984. Light trap collections of C. arboricola were variable throughout 1983 and 1984, but were essentially unimodal

with several peaks between April and November of each year. Culicoides arboricola was captured rarely in Bennett traps and had only 1 major peak in biting activity in May, 1984 (Figure 18).

Culicoides knowltoni was primarily a late-spring, early-summer species, with major peaks in abundance and biting activity between April and July, 1983, and May and June, 1984. Fall peaks in biting activity and abundance occurred in October, 1984.

Estimation of Prevalence

Between May, 1982, and April, 1983, unengorged specimens of Culicoides captured in Bennett traps at Paynes Prairie were dissected and examined for sporozoites in the salivary glands. Seventeen of 17 specimens of C. nanus, 9 of 9 C. crepuscularis, 33 of 33 C. arboricola and 12 of 12 C. hinmani were negative for salivary gland sporozoites. Sporozoites were found in a single, unengorged specimen of C. edeni, captured on 25 August, 1982, at Site A. Dissections of 209 other unengorged specimens of C. edeni, collected between May and April were negative. Unfortunately, an uninfected recipient turkey was not available for inoculation of the sporozoites to confirm their identification.

Between April, 1983, and May, 1984, unengorged specimens of Culicoides captured at Paynes Prairie and Fisheating Creek were pooled and inoculated into domestic turkey poults. At Paynes Prairie, 2 of 9 pools of C. edeni, totaling 343 individuals, were positive for H. meleagridis, resulting in an estimated minimum yearly prevalence of 0.58% (Table 8). The positive pools were collected in November, 1983, and May, 1984, during periods of active, natural transmission of the parasite.

At Fisheating Creek, 17 of 49 pools of C. edeni, totaling 816 individuals, were positive for H. meleagridis, resulting in an estimated minimum yearly prevalence of 2.08% (Table 9). Positive pools were collected in April, July, September and November, 1983, and February, March and May, 1984, during active natural transmission of the parasite.

Unengorged individuals of other species of Culicoides were not captured in sufficient numbers to make regular attempts at isolation. Pools of C. hinmani, C. arboricola and C. knowltoni, collected at unequal intervals throughout the year were negative for H. meleagridis (Table 10).

Table 2. New Jersey light trap collections - Paynes Prairie
May 1982 - July 1984

	Site A*	Site B#	Site A + B
Species	Total (%)	Total (%)	Total (%)
<i>C. insignis</i>	1,501 (14.3%)	17,495 (73.3%)	18,996 (55.3%)
<i>C. edeni</i>	2,693 (25.7%)	825 (3.5%)	3,518 (10.2%)
<i>C. stellifer</i>	1,474 (14.1%)	1,513 (6.3%)	2,987 (8.7%)
<i>C. arboricola</i>	1,706 (16.3%)	1,070 (4.5%)	2,776 (8.1%)
<i>C. crepuscularis</i>	915 (8.7%)	475 (2.0%)	1,390 (4.1%)
<i>C. spinosus</i>	184 (1.8%)	919 (3.9%)	1,103 (3.2%)
<i>C. scanloni</i>	225 (2.1%)	471 (2.0%)	696 (2.0%)
<i>C. nanus</i>	606 (5.8%)	73 (0.3%)	679 (2.0%)
<i>C. debilipalpus</i>	416 (4.0%)	90 (0.4%)	506 (1.5%)
<i>C. niger</i>	120 (1.1%)	363 (1.5%)	483 (1.4%)
<i>C. villosipennis</i>	152 (1.5%)	106 (0.4%)	258 (0.8%)
<i>C. hinmani</i>	148 (1.4%)	95 (0.4%)	243 (0.7%)
<i>C. paraensis</i>	130 (1.2%)	41 (0.2%)	171 (0.5%)
<i>C. ousaifani</i>	61 (0.6%)	39 (0.2%)	100 (0.3%)
<i>C. venustus</i>	5 (<0.1%)	89 (0.4%)	94 (0.3%)
<i>C. bauei</i>	33 (0.3%)	37 (0.2%)	70 (0.2%)
<i>C. bickleyi</i>	25 (0.2%)	45 (0.2%)	70 (0.2%)
<i>C. haematopodus</i>	40 (0.4%)	23 (0.1%)	63 (0.2%)
<i>C. alachua</i>	1 (<0.1%)	44 (0.2%)	45 (0.1%)
<i>C. biguttatus</i>	19 (0.2%)	19 (0.1%)	38 (0.1%)
<i>C. tissoi</i>	12 (0.1%)	18 (0.1%)	30 (0.1%)
<i>C. guttipennis</i>	8 (0.1%)	9 (<0.1%)	17 (0.1%)
<i>C. pechumani</i>	3 (<0.1%)	0 (0.0%)	3 (<0.1%)
<i>C. furens</i>	0 (0.0%)	2 (<0.1%)	2 (<0.1%)
<i>C. mulrennani</i>	0 (0.0%)	2 (<0.1%)	2 (<0.1%)
<i>C. chiopterus</i>	1 (<0.1%)	0 (0.0%)	1 (<0.1%)
<i>C. piliferus</i>	0 (0.0%)	1 (<0.1%)	1 (<0.1%)
Total	23,864	10,478	34,342

* 52 nights of trapping

44 nights of trapping

Table 3. Engorged specimens of Culicoides captured in Bennett traps at Paynes Prairie, May 1982 - July 1984.

	Site A*	Site B#	Site A + B
Species	Total (%)	Total (%)	Total (%)
<u>C. edeni</u>	292 (42.7%)	393 (52.8%)	685 (48.0%)
<u>C. hinmani</u>	205 (30.0%)	169 (22.7%)	374 (26.2%)
<u>C. scanloni</u>	21 (3.1%)	92 (12.4%)	113 (7.9%)
<u>C. arboricola</u>	74 (10.8%)	34 (4.6%)	108 (7.6%)
<u>C. nanus</u>	58 (8.5%)	15 (2.0%)	73 (5.1%)
<u>C. baueri</u>	12 (1.8%)	19 (2.6%)	31 (2.2%)
<u>C. paraensis</u>	6 (0.9%)	10 (1.3%)	16 (1.1%)
<u>C. haematopodus</u>	7 (1.0%)	7 (0.9%)	14 (1.0%)
<u>C. crepuscularis</u>	6 (0.9%)	3 (0.4%)	9 (0.6%)
<u>C. guttipennis</u>	2 (0.3%)	1 (0.1%)	3 (0.2%)
<u>C. insignis</u>	0 (0.0%)	1 (0.1%)	1 (0.1%)
<u>C. ousairani</u>	1 (0.1%)	0 (0.0%)	1 (0.1%)
Total	684	744	1,428

* Traps operated on 56 evenings for a total of 113 hours

Traps operated on 49 evenings for a total of 98 hours

Table 4. New Jersey light trap collections - Fisheating Creek, December 1982 - November 1984. Total catch was made on 28 different nights of trapping.

Species	Total	% Total
<u>C. insignis</u>	7,940	44.5
<u>C. edeni</u>	7,279	40.8
<u>C. knowltoni</u>	1,356	7.6
<u>C. stellifer</u>	742	4.2
<u>C. arboricola</u>	287	1.6
<u>C. hinmani</u>	129	0.7
<u>C. pusillus</u>	60	0.3
<u>C. ousaieani</u>	28	0.2
<u>C. baueri</u>	15	0.1
<u>C. paraensis</u>	14	0.1
<u>C. debilipalpus</u>	4	0.02
<u>C. haematopotus</u>	2	0.01
<u>C. bickleyi</u>	1	0.01
Total	17,857	100.00

Table 5. Engorged specimens of Culicoides captured in Bennett traps at Fisheating Creek, December 1982 - November 1984. Traps were operated on 47 different evenings for a total of 108 hours.

Species	Total	% Total
<u>C. edeni</u>	2,038	79.6
<u>C. hinmani</u>	475	18.6
<u>C. knowltoni</u>	35	1.4
<u>C. arboricola</u>	12	0.5
<u>C. baueri</u>	1	0.04
Total	2,561	100.0

Table 6. Susceptibility of wild-caught specimens of Culicoides to Haemoproteus meleagridis. Fractions represent number positive/ number examined and are followed by percent of total.

Development of Parasite			
Species	None	Partial*	Complete**
<u>C. edeni</u>			32/52 (61.5%)
<u>C. arboricola</u>			6/28 (21.4%)
<u>C. haematopotus</u>			1/6 (16.7%)
<u>C. hinmani</u>			8/72 (11.1%)
<u>C. knowltoni</u>			1/14 (7.1%)
<u>C. paraensis</u>		1/2 (50.0%)	
<u>C. nanus</u>		8/27 (29.6%)	
<u>C. scanloni</u>		3/25 (12.0%)	
<u>C. baueri</u>		2/24 (8.3%)	
<u>C. crepuscularis</u>	3/3 (100%)		

* Degenerating oocysts present

** Invasion of salivary glands by sporozoites

Table 7. Mean capture times for specimens of Culicoides taken in Bennett traps at Paynes Prairie and Fisheating Creek. Values are in minutes, relative to nautical sunset. Numbers in parentheses are standard deviations.

Culicoides Species - Paynes Prairie

Quarter+	<u>hinmani</u>	<u>edeni</u>	<u>arboricola</u>
1		18.7a (26.9)	26.3a (22.0)
2	-35.3a (28.1)	15.1b (21.4)	8.1c (31.2)
3	-28.0a (30.8)	12.0b (27.9)	17.3b (20.0)
4	-24.6a (24.1)	14.8b (22.5)	40.4c (20.4)

Culicoides Species - Fisheating Creek

Quarter	<u>hinmani</u>	<u>edeni</u>	<u>arboricola</u>	<u>knowltoni</u>
1	-33.3a (22.8)	5.1b (29.1)	21.7bc* (9.1)	51.5c* (4.9)
2	-58.6a (32.9)	0.0b (35.3)	21.3c (31.1)	26.9c (18.2)
3	-49.3a (27.6)	6.4b (32.9)	24.8b* (41.4)	26.5b (16.8)
4	-23.9a (27.8)	14.5b (29.4)	55.0c* (19.1)	31.7bc (20.4)

a Values with the same letter are not significantly different, $P < 0.05$

* N = 10

+ Quarter 1 = January-March; Quarter 2 = April-June;
Quarter 3 = July-September; Quarter 4 = October-December

Table 8. Yearly prevalence of Haemoproteus meleagridis in specimens of Culicoides edeni at Paynes Prairie.

Month	Year	Pools	# Flies	Isolations
April	1983	1	35	0
June	1983	1	83	0
July	1983	1	19	0
August	1983	1	10	0
November	1983	1	20	1
March	1984	1	12	0
April	1984	1	12	0
May	1984	2	152	1
Total:		9	343	2
Minimum Yearly Prevalence:			0.58%	

Table 9. Yearly prevalence of Haemoproteus meleagridis in specimens of Culicoides edeni at Fisheating Creek.

Month	Year	Pools	# Flies	Isolations
April	1983	2	98	2
May	1983	2	23	0
June	1983	1	17	0
July	1983	4	99	3
August	1983	2	42	0
September	1983	2	56	2
November	1983	7	101	1
December	1983	2	81	0
January	1984	1	9	0
February	1984	4	42	1
March	1984	9	117	5
April	1984	7	75	1
May	1984	6	56	2
Total:		49	816	17
Minimum Yearly Prevalence:			2.08%	

Table 10. Attempted isolations of Haemoproteus meleagridis from pools of Culicoides hinmani, Culicoides arboricola and Culicoides knowltoni at Paynes Prairie (PAP) and Fisheating Creek (FEC).

Culicoides hinmani

Location	Month	Year	Pools	# Flies	Isolations
PAP	July	1983	1	3	0
PAP	August	1983	1	4	0
PAP	September	1983	1	8	0
FEC	May	1983	1	10	0
FEC	August	1983	1	2	0
FEC	March	1984	1	7	0
FEC	April	1984	1	5	0

Culicoides arboricola

Location	Month	Year	Pools	# Flies	Isolations
PAP	April	1983	1	10	0
PAP	May	1983	1	13	0
PAP	March	1984	1	5	0
FEC	April	1984	1	8	0

Culicoides knowltoni

Location	Month	Year	Pools	# Flies	Isolations
FEC	June	1983	1	3	0
FEC	July	1983	1	4	0
FEC	September	1983	1	4	0
FEC	May	1984	2	53	0

- Figure 1. Ookinete of Haemoproteus meleagridis from the midgut of a specimen of Culicoides edeni, 24 hours after the fly engorged on an infected turkey. A mass of pigment (arrow) is located near the posterior end of the organism. Bar = 10 μ m. Note: Figures 1-6 were taken with Normarski contrast interference microscopy.
- Figure 2. Developing oocysts (arrows) of Haemoproteus meleagridis on the midgut of a specimen of Culicoides edeni, 4 days after the fly had taken a blood meal from an infected turkey. Bar = 50 μ m.
- Figure 3. A 6-day-old, degenerating oocyst of Haemoproteus meleagridis from a specimen of Culicoides edeni. The oocyst contains large refractile granules (arrow). Bar = 10 μ m.
- Figure 4. A mature, 6-day-old oocyst of Haemoproteus meleagridis from a specimen of Culicoides edeni. The oocyst is packed with slender sporozoites that are parallel to one another. A small, refractile residual body (arrow) is present. Bar = 10 μ m.
- Figure 5. One of the 2 salivary glands from a specimen of Culicoides edeni that had engorged on an infected turkey 7 days earlier. The gland consists of an elongate primary lobe and several smaller, apical lobes. The shadows of sporozoites are barely visible in secretory cells composing the primary lobe (arrows). Bar = 10 μ m.
- Figure 6. A crushed salivary gland from a specimen of Culicoides edeni that had engorged on an infected turkey 7 days earlier. Numerous elongate sporozoites (arrows) are located within the secretory cells. Bar = 10 μ m.

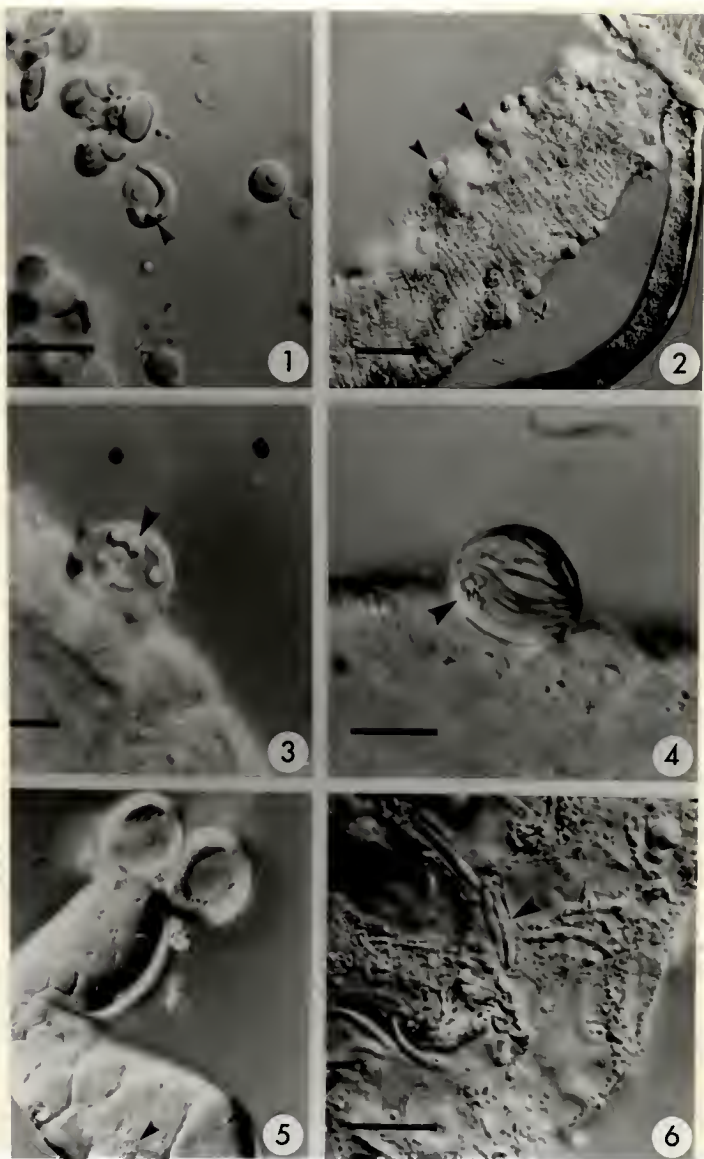


Figure 7. Scatter plot of capture times for specimens of Culicoides hinmani that were captured in Bennett traps at Paynes Prairie and Fisheating Creek. Capture time is plotted as minutes before or after nautical sunset (reference line). Arrow indicates mean capture time.

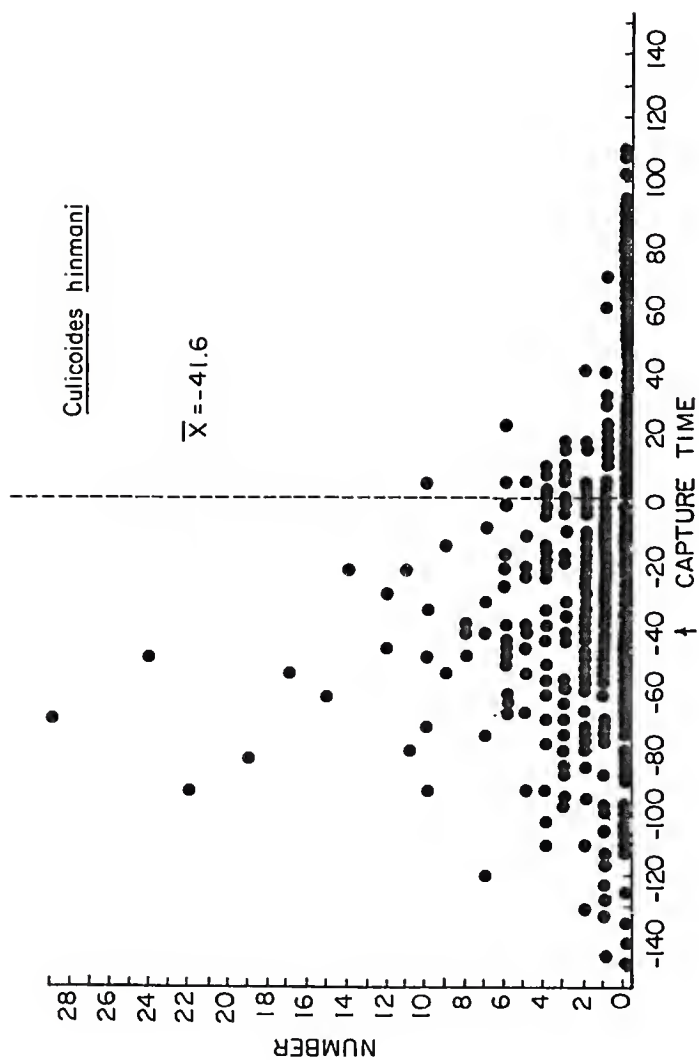


Figure 8. Scatter plot of capture times for specimens of Culicoides edeni that were captured in Bennett traps at Paynes Prairie and Fisheating Creek. Capture time is plotted as minutes before or after nautical sunset (reference line). Arrow indicates mean capture time.

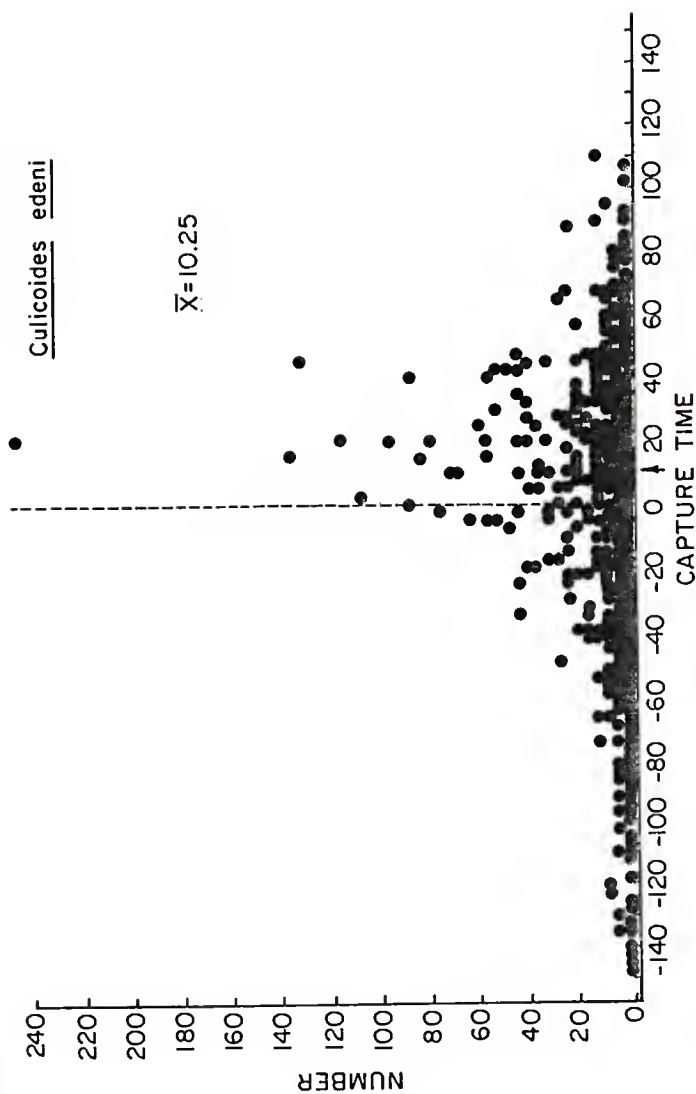


Figure 9. Scatter plot of capture times for specimens of Culicoides arboricola that were captured in Bennett traps at Paynes Prairie and Fisheating Creek. Capture time is plotted as minutes before or after nautical sunset (reference line). Arrow indicates mean capture time.

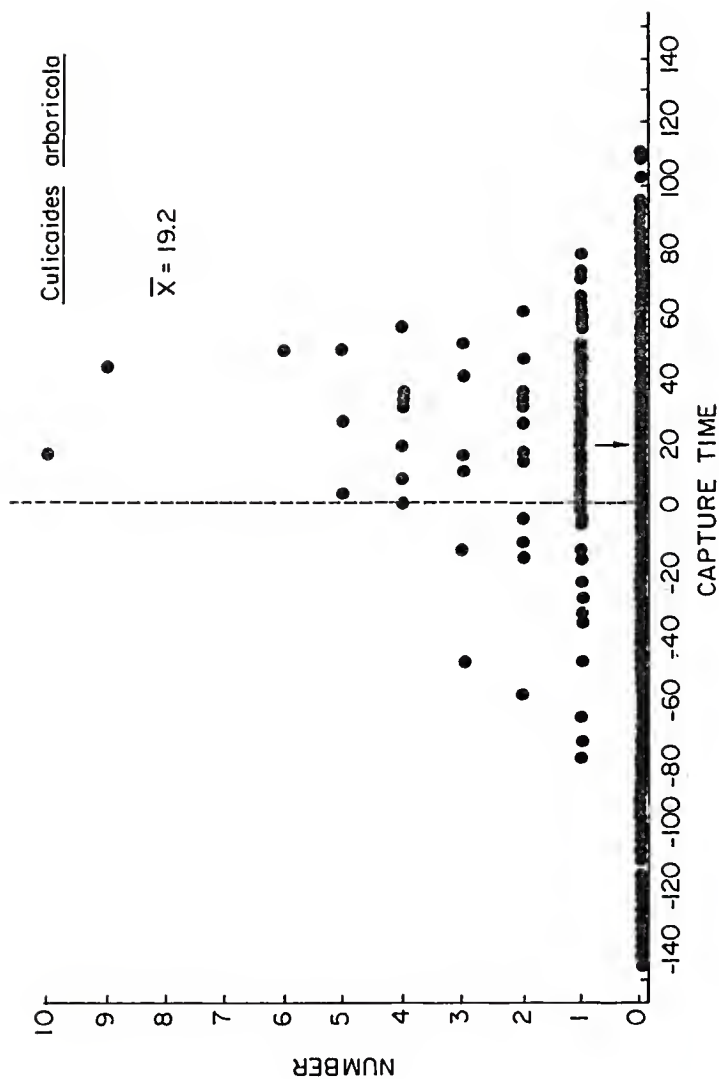


Figure 10. Scatter plot of capture times for specimens of Culicoides knowltoni that were captured in Bennett traps at Paynes Prairie and Fisheating Creek. Capture time is plotted as minutes before or after nautical sunset (reference line). Arrow indicates mean capture time.

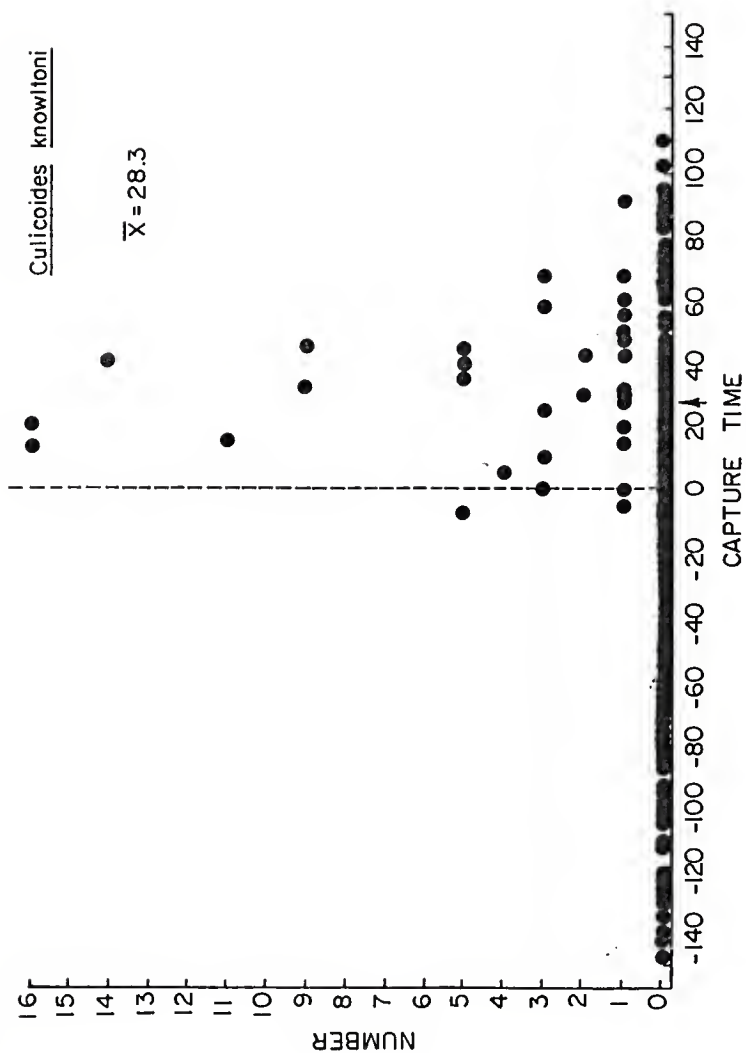
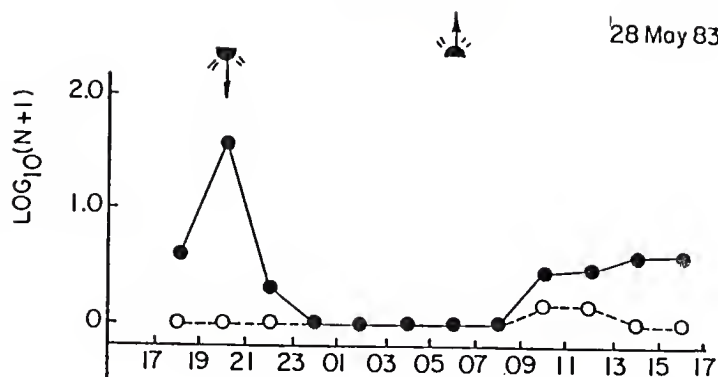


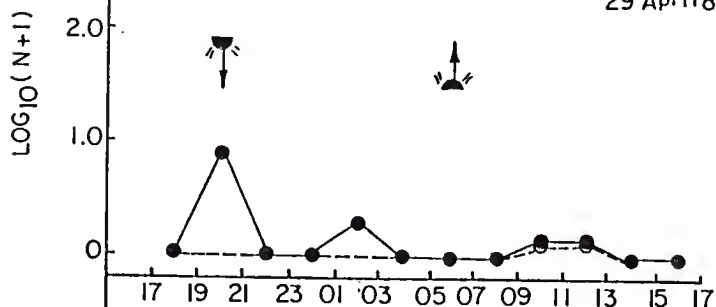
Figure 11. Modified New Jersey suction trap catches of specimens of Culicoides edeni at Fisheating Creek during the March, April and May, 1983 collecting trips. (●—● = canopy trap; ○—○ = ground trap). Rising and setting suns indicate the 2-hour sampling period that included dawn or dusk.

Culicoides edeni

28 May 83



29 April 83



31 March 83

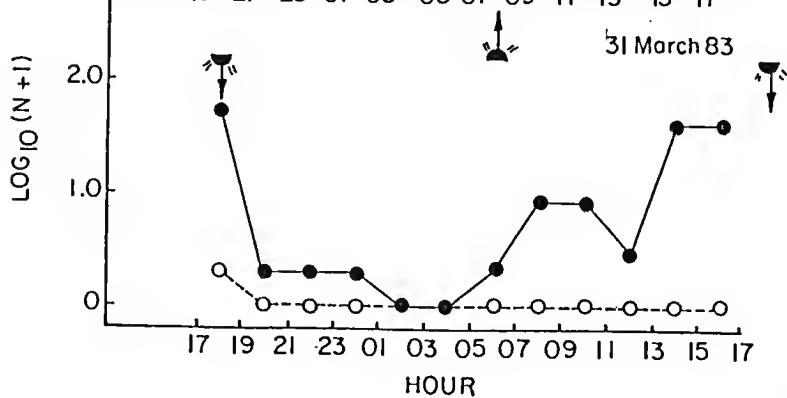


Figure 12. Modified New Jersey suction trap catches of specimens of Culicoides hinmani at Fisheating Creek during the March, April and May, 1983 collecting trips. (●—● = canopy trap; ○--○ = ground trap). Rising and setting suns indicate the 2-hour sampling period that included dawn or dusk.

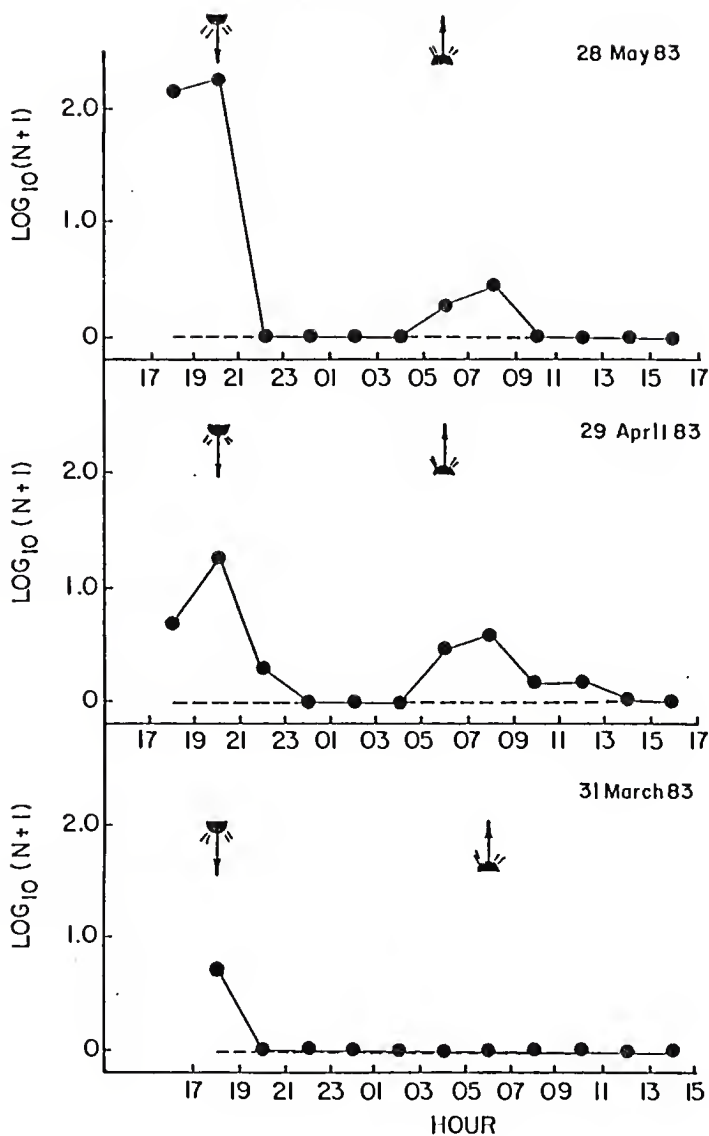
Culicoides hinmani

Figure 13.

Transmission vs. abundance of 3 species of Culicoides at Site A at Paynes Prairie that were able to support development of Haemoproteus meleagridis. (●—● = Bennett trap catch; ○—○ = New Jersey light trap catch). The kite diagram at the top of the figure indicates the % of sentinel birds that became infected with Haemoproteus meleagridis during 4-week periods between May, 1982 and June, 1984. Blank areas in the diagram indicate periods where transmission did not occur. Marks on the x-axis that follow each month indicate the middle of that month.

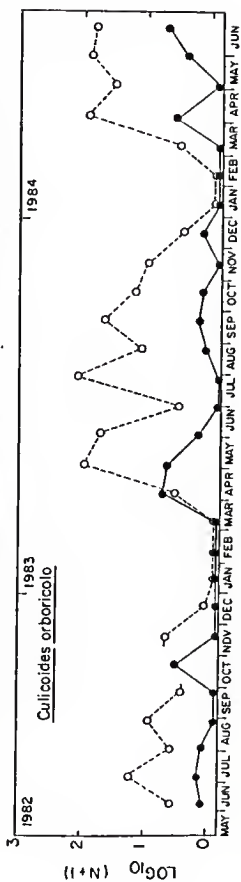
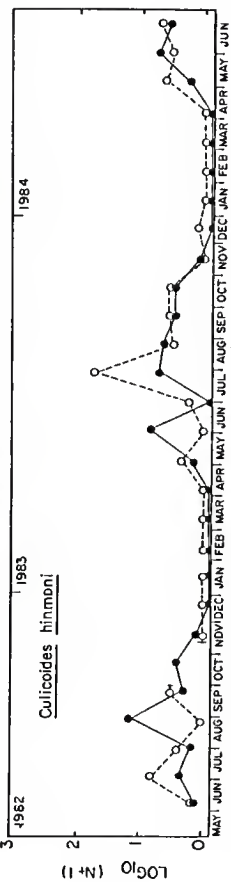
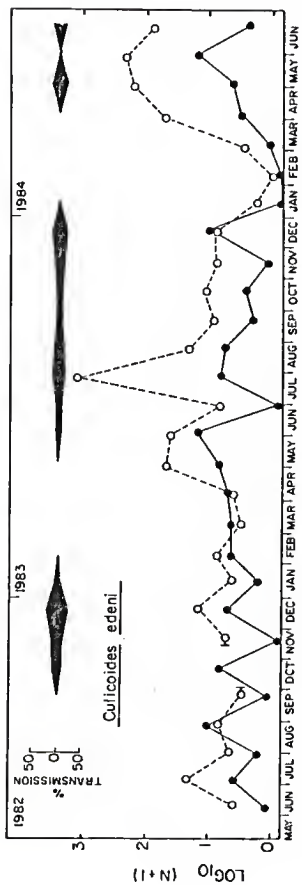


Figure 14.

Transmission vs. abundance of 3 species of *Culicoides* at Site B at Paynes Prairie that were able to support development of *Haemoproteus meleagridis*. (●—● = Bennett trap catch; ○—○ = New Jersey light trap catch). The kite diagram at the top of the figure indicates the % of sentinel birds that became infected with *Haemoproteus meleagridis* during 4-week periods between July, 1982 and June, 1984. Blank areas in the diagram indicate periods where transmission did not occur. Marks on the x-axis that follow each month indicate the middle of that month.

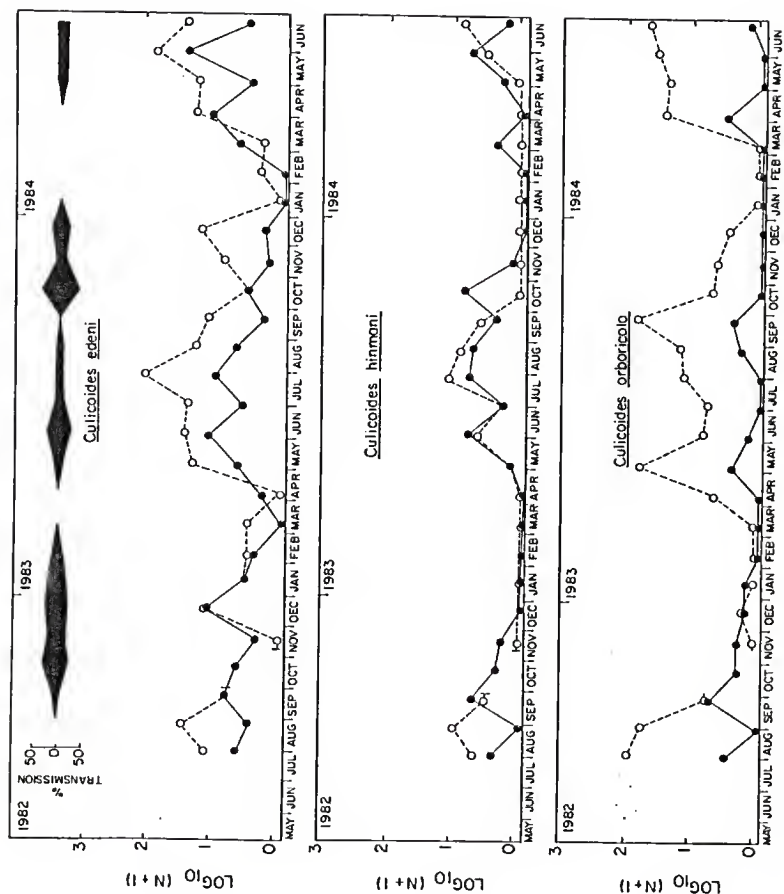


Figure 15. Departures from normal for average monthly temperatures during 1982, 1983 and 1984 at Paynes Prairie. Normals are based on 30 year averages for each month between 1951 and 1980 (Climatological Data: Florida, 1982, 1983, 1984).

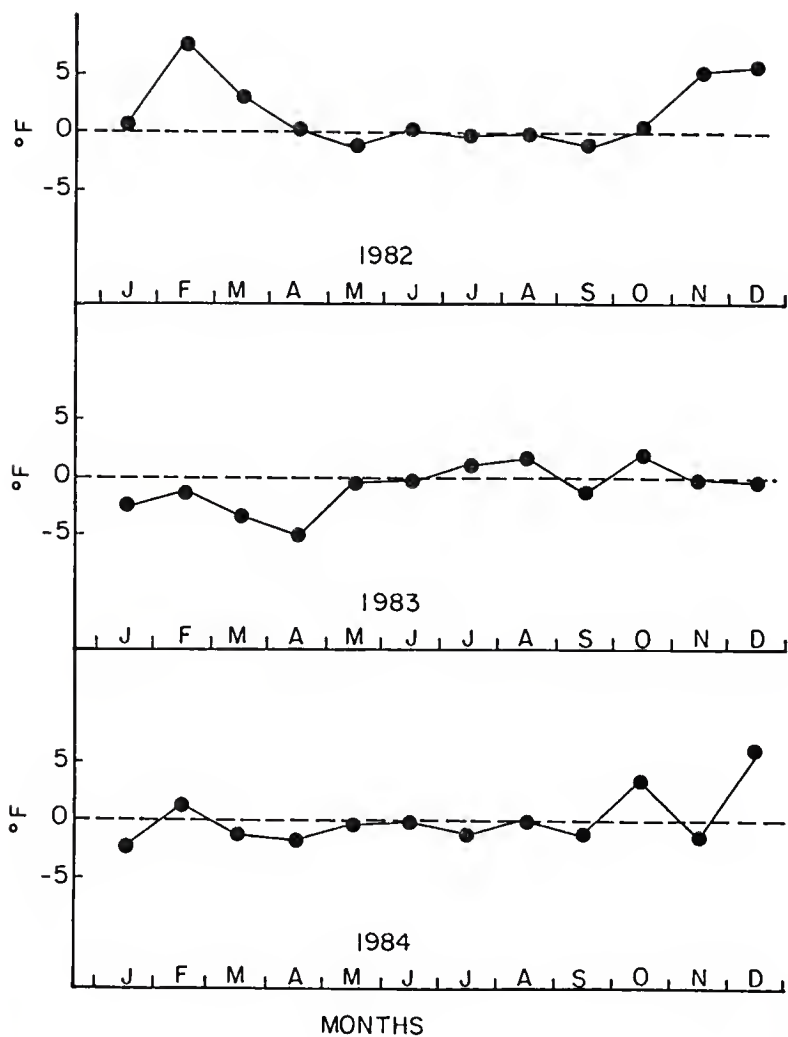


Figure 16. Departures from normal for total monthly precipitation during 1982, 1983 and 1984 at Paynes Prairie. Normals are based on averages of monthly totals between 1951 and 1980 (Climatological Data: Florida, 1982, 1983, 1984).

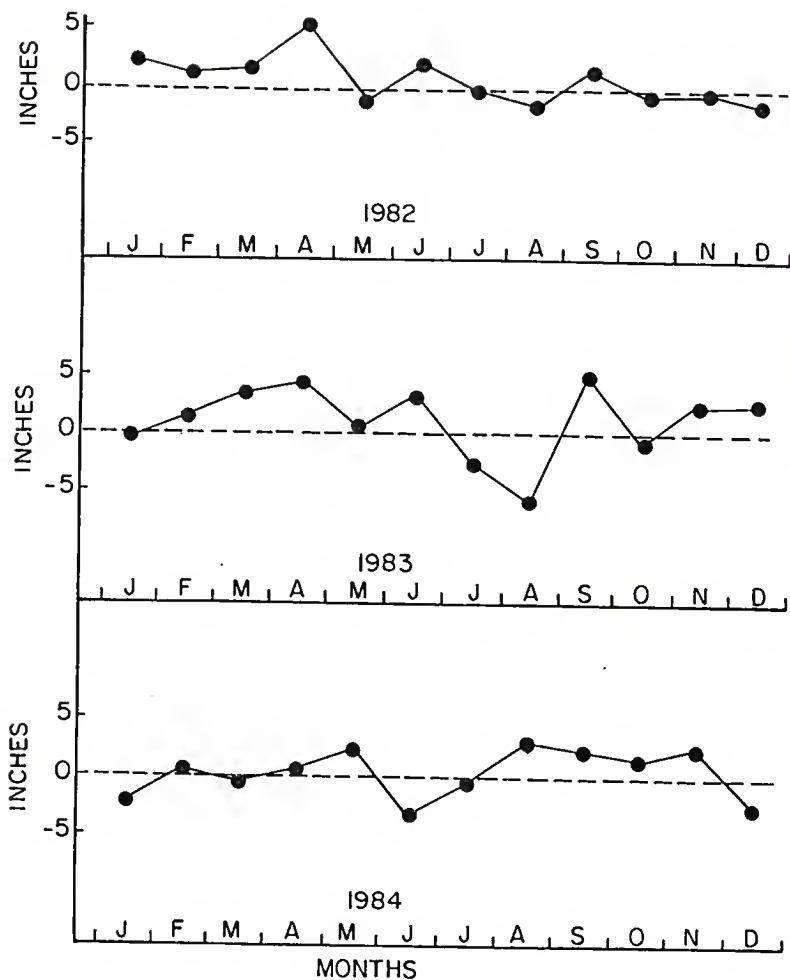


Figure 17. Average monthly temperatures at Paynes Prairie (●—●) and Fisheating Creek (○--○) during 1982, 1983 and 1984 (Climatological Data: Florida, 1982, 1983, 1984).

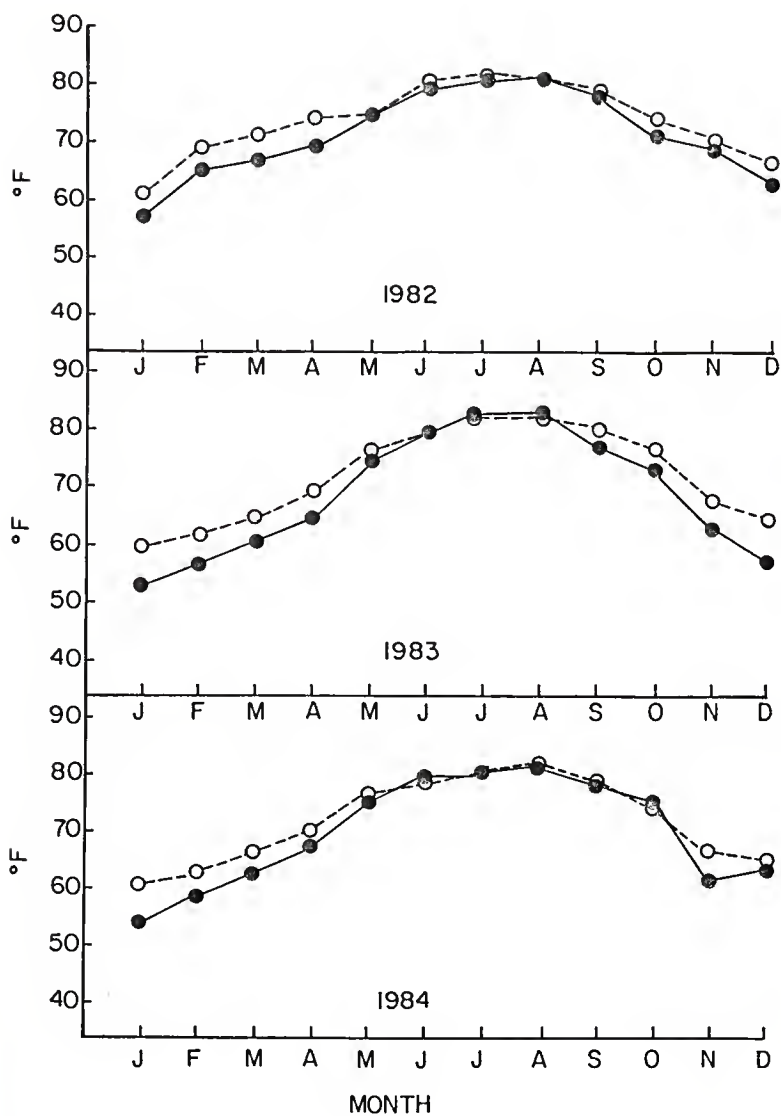


Figure 18. Transmission vs. abundance of 4 species of Culicoides at Fisheating Creek that were able to support development of Haemoproteus meleagridis. (●—● = Bennett trap catch; ○—○ = New Jersey light trap catch). The kite diagram at the top of the figure indicates the % of sentinel birds that became infected with Haemoproteus meleagridis during monthly collecting trips between March, 1983 and September, 1984. Sentinels were not exposed during the October, 1984 trip. None of the sentinels exposed during the November, 1984 collecting trip became infected. Marks on the x-axis that follow each month mark the middle of that month.

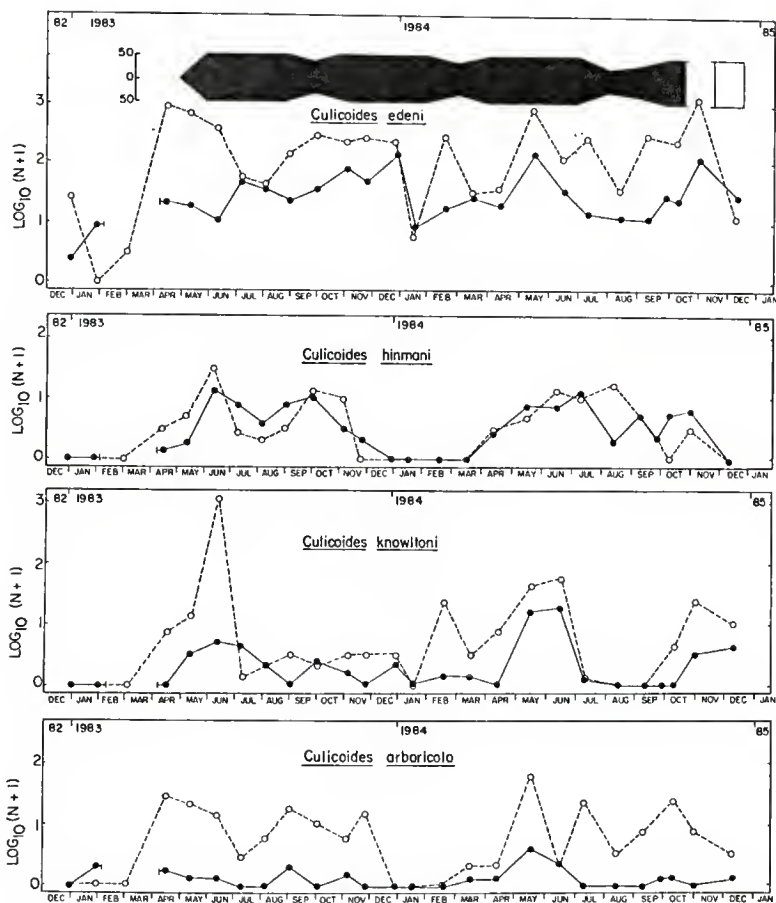


Figure 19. Departures from normal for average monthly temperatures during 1982, 1983 and 1984 at Fisheating Creek. Normals are based on 30 year averages each month between 1951 and 1980 (Climatological Data: Florida, 1982, 1983, 1984).

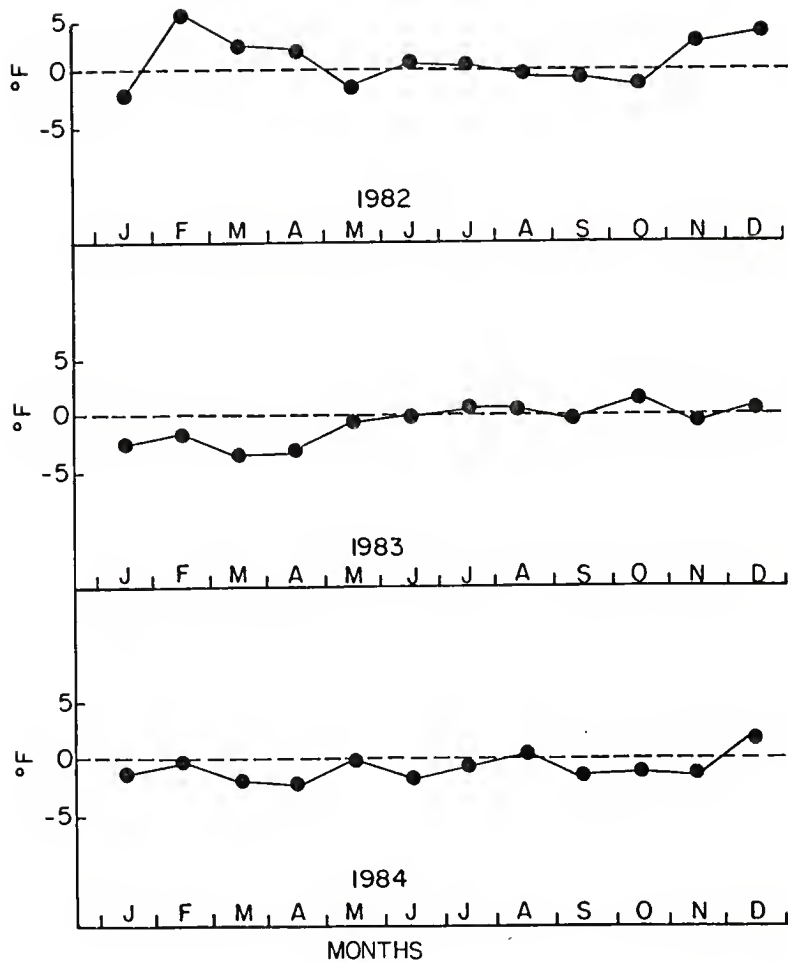
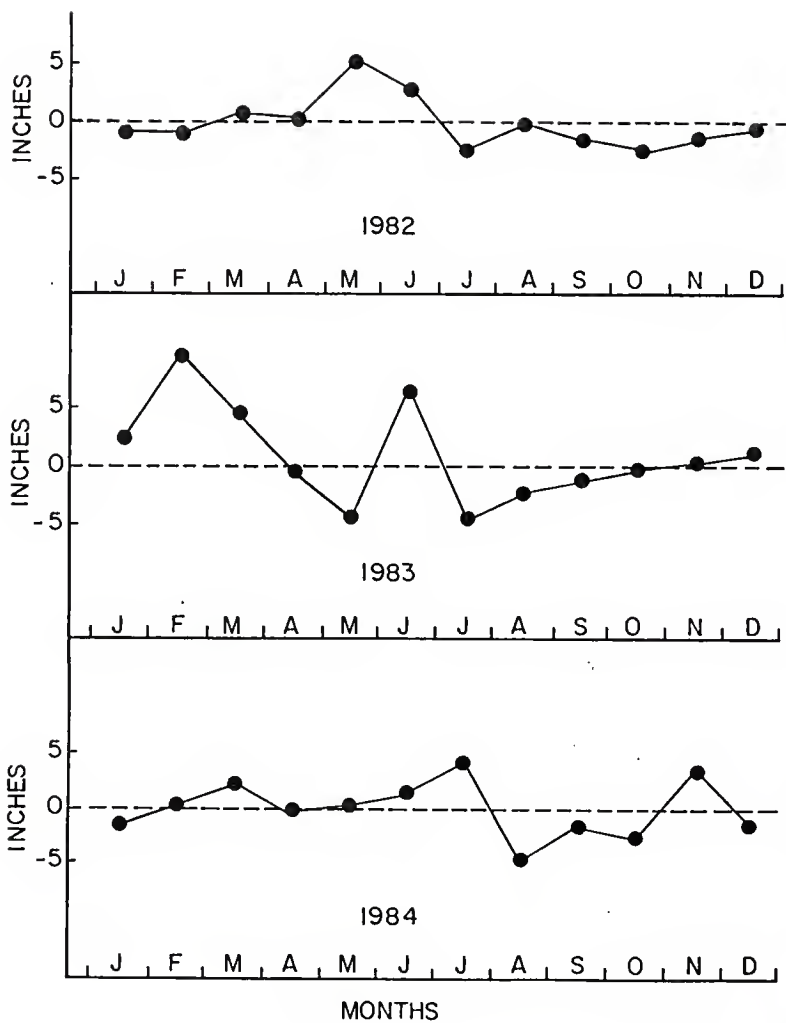


Figure 20. Departures from normal for total monthly precipitation during 1982, 1983 and 1984 at Fisheating Creek. Normals are based on averages of monthly totals between 1951 and 1980 (Climatological Data: Florida, 1982, 1983, 1984).



PathogenicityExperiment 1 - Pathology

Gross observations - spontaneous deaths. As early as 7 days post-infection (DPI) a number of subtle, behavioral changes became evident among poult within the high dose group. The birds stood with slightly drooped wings, ruffled feathers and partially or completely closed eyes and were less active than birds belonging to either the low dose or control groups. Between 7 and 14 DPI, birds in the high dose group developed a mild diarrhea which produced a "pasty" vent. Their physical condition continued to deteriorate and by 15 DPI, most birds exhibited lameness in 1 or both legs and severe depression. Most were emaciated, dehydrated and anorexic.

On days 19, 20 and 22 DPI, 4 birds (33%) in the high dose group died. The deaths occurred from 2 to 5 days following the appearance of young gametocytes in the peripheral circulation. At necropsy, the skeletal muscles contained numerous fusiform, white cysts, up to 1.0 mm in length and 0.5 mm in diameter. They were scattered diffusely throughout the skeletal muscles, but were most common in the pectoral muscles. All were oriented parallel to the muscle fibers. From 30 to 50% of the cysts were discolored red by hemorrhage (Figure 21).

The 4 birds had a number of secondary lesions unrelated to the muscle cysts. One bird had a large white nodule, approximately 5 mm in diameter, that occupied the posterior portion of 1 lung. Another bird had thickened air sacs with scattered white plaques that were about 5 mm in diameter. In all 4 birds, portions of the gut were swollen and flaccid and the mucosa had multifocal areas of discoloration. Reddish or black, tarry mucoid material was occasionally present in the jejunum. A bacterial culture from 1 bird was positive for Salmonella enteriditis Group B.

A randomly selected bird in the control group was killed and necropsied at 22 DPI to determine whether the cysts observed in the birds that died may have resulted from contamination with Sarcocystis. Tissue cysts were not evident in any skeletal muscles of this bird. All organs and tissues were grossly normal.

At 27 DPI, cloacal swabs from 2 of 3 randomly selected low dose birds, 2 of 3 randomly selected control birds and 3 of 5 randomly selected high dose birds were positive for Salmonella enteriditis Group B, serotype heidelberg.

Gross observations - surviving birds. At 8 weeks post-infection, all surviving birds were necropsied. Nine of the 12 low dose birds and 8 of the 8 high dose birds had low numbers of fusiform, white cysts in the pectoral

muscles. They were 2-3 times larger and were more diffuse than cysts observed in the 4 fatal infections. Cysts were not detected in any of the 11 control birds.

All surviving birds had focal areas of reddened, swollen mucosa and occasional petechial hemorrhages throughout the length of the gut. None of the birds exhibited clinical signs of salmonellosis, i.e. "pasty" vent, bloody diarrhea or depression. The "pasty vents" observed in high dose birds at 1 week PI resolved spontaneously in surviving birds by 4 weeks PI. At the termination of the experiment, cloacal swabs from 2 of 11 control birds, 1 of 12 low dose birds and 1 of 8 high dose birds were positive for Salmonella enteritidis Group B. Coccidian oocysts were not detected in pooled fecal samples at either 4 or 8 weeks post-infection.

Incidental findings included the presence of numerous white nodules, approximately 5 mm in diameter, that were scattered throughout the mesentery lining the abdominal cavity of 1 control bird and 2 high dose birds.

Average wet weights of hearts and livers removed from the birds at necropsy were not significantly different for any group (Table 11). The difference in average spleen weights among the 3 groups was highly significant ($p < .0002$).

Table 11. Average organ* weights at necropsy

Group	N	Heart (p=0.112)	Liver (p=0.0641)	Spleen (p=0.0002)
Control	11	0.443	1.70	0.093
Low	12	0.457	1.74	0.130
High	8	0.489	1.93	0.163

* Weights expressed as % of total body weight at necropsy

Microscopic observations - spontaneous deaths. The most significant lesions found in tissues from the 4 spontaneous deaths were in the skeletal muscles. All 4 birds had numerous intact and degenerating, fusiform megaloschizonts between the muscle fibers. The schizonts ranged from 43 to 155 μm in diameter with a mean diameter of 95.0 μm ($n = 35$, $SD = 34.67$). Depending on the plane of section, they ranged up to 600 μm in length. Most megaloschizonts were oriented parallel to the muscle fibers, although a few were perpendicular. Many were outlined by a thick, hyaline wall which occasionally indented slightly to form node-like constrictions (Figures 22, 23).

Mature megaloschizonts were packed with small, spherical merozoites less than 1 μm in diameter. Each contained a small mass of chromatin. Some immature megaloschizonts contained irregularly shaped cytomeres of different sizes that were located next to the cyst wall. Chromatin masses were arranged around the periphery of each cytomere. Other megaloschizonts were packed with round, granular, dark blue cytomeres. As megaloschizonts matured, the number of cytomeres increased in inverse proportion to their diameter. Merozoites developed as buds from the outer surface of the cytomeres.

A severe hemorrhagic myositis was associated with the megaloschizonts. Ruptured schizonts were surrounded by

a mixed inflammatory infiltrate composed of macrophages, heterophils, giant cells and red blood cells (Figures 23, 28). They were frequently invaded by macrophages and heterophils. Macrophages were often adjacent to the outer wall of ruptured megaloschizonts and occasionally were seen phagocytizing merozoites that had been liberated (Figures 24, 25). Giant cells commonly were found adjacent to intact and degenerating cysts (Figures 23, 25). Muscle fibers adjacent to the cysts were swollen, rounded and hyaline and often contained small, gray to dark blue granules which were oriented occasionally into parallel lines (Figures 26, 27). Dark blue calcium deposits, visible with hematoxylin and eosin as well as with von Kossa's calcium stain, occupied much of the cytoplasm of muscle fibers adjacent to more degenerate cysts (Figures 26, 27).

Capillaries and venules adjacent to or near degenerating megaloschizonts were often occluded partially or completely by thrombi composed of pink staining, fibrinous material (Figure 29). Giant cells often surrounded thrombi or were adjacent to occluded vessels.

Liver and spleen sections from the 2 birds that died spontaneously at 22 DPI, 5 days after gametocytes first appeared in circulating red cells, had numerous golden yellow pigment deposits in the cytoplasm of macrophages (Figure 30). The 2 birds that died on 19

and 20 DPI lacked similar deposits. Spleen sections from all 4 birds had extensive areas of follicular hypoplasia caused by a large reduction in the size of the periaarterial lymphatic sheaths. Numerous mature and ruptured schizonts were present in reticular cells in the spleen of 1 bird. These schizonts were smaller in diameter and lacked the thick, hyaline wall that surrounded megaloschizonts from muscle tissue (Figures 31, 32). Most averaged from 10 to 15 μ m in diameter and contained small, spherical zoites, less than 1 μ m in diameter. A few contained elongate zoites.

Heart sections from 1 bird had several thrombi with associated giant cells and a single, large megaloschizont.

Incidental findings in lung tissue from 3 of the 4 birds included the presence of large granulomas composed of giant cells, mononuclear cells and heterophils that surrounded large, amorphous eosinophilic central cores. Fungal hyphae resembling Aspergillus sp. were evident in sections from 1 bird. Epithelial cells lining alveolar capillaries were hypertrophic and associated capillaries were congested. A fibrino-hemorrhagic exudate containing macrophages and heterophils flooded alveolar spaces. Some large blood vessels were occluded by thrombi. Sections of air sacs of 1 bird and mesentery of another had similar granulomas that surrounded eosinophilic masses containing fungal hyphae.

Focal areas of enteritis characterized by the presence of heterophils in the lamina propria and submucosa were present in sections of intestine and cecum. Coccidian parasites were not detected. Sections of kidney, brain, bone marrow, proventriculus and gizzard were unremarkable.

Microscopic observations - surviving birds. At 8 weeks post-infection, nodular infiltrates of mononuclear cells, macrophages, heterophils and giant cells were evident in sections of skeletal muscle from low dose and high dose birds (Figure 33). The hyaline remnants of the outer wall of degenerating megaloschizonts and necrotic and calcified muscle fibers were at the center of some of the nodules. A scattered lymphocytic, heterophilic infiltrate was frequently perivascular and often present between muscle fibers. Pectoral muscle from 1 low dose bird contained a degenerating cyst with merozoites (Figure 34). Thrombi surrounded by macrophages and heterophils were present in some sections (Figure 34). Remnants of degenerating muscle fibers, infiltrated with macrophages and heterophils, were scattered randomly throughout the sections of muscle (Figure 35).

Sections of liver, lung and spleen from both low and high dose birds contained moderate to extensive, random deposits of pigment. No pigment was found in control birds. Deposits in the liver and spleen were massive

and brownish-black. Those in the lung were smaller and golden-brown. All were contained in macrophages. Follicular hyperplasia was common in the spleens of all infected birds. The degree of hyperplasia as well as the number of pigment deposits varied directly with the size of the infective dose.

Sections of gut from the control, low dose and high dose birds had a few multifocal areas of infiltrate composed of heterophils. Coccidian parasites were not observed. One high dose bird had a granulomatous peritonitis. The granulomas were composed of macrophages, heterophils and giant cells that surrounded amorphous, eosinophilic masses.

Sections of brain, bone marrow, kidney, heart, proventriculus and gizzard were unremarkable.

Parasitemia. Young gametocytes appeared in the peripheral circulation of all birds in the high and low dose groups at 17 DPI. The control birds remained uninfected throughout the study. The parasitemia in both infected groups quickly reached a peak by 21 DPI and then rapidly fell within 7 days to values less than 10% of those at the crisis (Figure 36). A second, smaller peak occurred at approximately 5 weeks post-infection. Both groups remained patent through the course of the study, although parasitemias were often less than 1.0 %.

At the crisis, the peak parasitemia in the high dose birds reached an average high of 5,760 gametocytes per 10,000 red cells. Two birds had peak parasitemias that exceeded 7,000 gametocytes per 10,000 red cells. In many birds, more than 50% of the red cells contained developing gametocytes. Multiple infections of red cells were common, with some cells containing as many as 6 gametocytes. At the crisis, low dose birds had an average peak parasitemia of 2,109 gametocytes per 10,000 red cells, less than half that of the high dose group.

Weight. Statistical analysis of the weight data revealed that all 4 variables in the model statement, i.e. treatment, subject(treatment), week, treatment*week, were highly significant ($p < .0001$).

When comparisons were made by week, all 3 groups were significantly different 1 week post-infection (PI) and at the crisis at 3 weeks PI. Weights of high dose birds were significantly lower than control and low dose birds during all other weeks. Other differences between the control and low dose groups were not significant (Figure 37).

When comparisons were made within groups, control and low dose birds had significant increases in weight at each week PI. By contrast, average weights for the high dose group increased during each week, but not significantly at

0, 1 and 2 weeks PI, at 1, 2 and 3 weeks PI and at 3 and 4 weeks PI (Figure 37).

Tarsometatarsal length. Statistical analysis of tarsometatarsal length revealed that all 4 variables in the model statement were highly significant ($p = .0001$). When comparisons were made by week, all 3 groups were significantly different at the crisis, at 3 weeks PI. The high dose group had average tarsometatarsal lengths that were significantly shorter than control and low dose birds at all other weeks PI. Other differences between the control and low dose groups were not significant (Figure 38).

When comparisons were made within group, all groups showed a significant increase in tarsometatarsal length for each week.

Hematocrit. Statistical analysis of hematocrit revealed that all variables in the model statement were highly significant ($p = .0001$) except treatment*week ($p = .6886$). Comparisons among the 3 experimental groups, averaged over all weeks, were not significant ($p = .1617$). Comparisons among weeks, averaged over all groups, showed no significant differences among weeks 0, 6 and 8, among weeks 3, 6 and 7, among weeks 1, 4 and 5 and between weeks 2 and 4 PI (Figure 39).

Plasma protein concentration. Statistical analysis of plasma protein concentration revealed that all variables in the model statement were significant ($p < .0125$).

When comparisons were made by week, all 3 groups were significantly different at 1 week PI. Control birds had the highest average plasma protein concentration and high dose birds had the lowest. By 2 weeks PI, average plasma protein concentrations were significantly greater for high dose birds than either low dose or control birds. The 3 experimental groups were not significantly different at 3 and 4 weeks PI. At 5 and 6 weeks PI, high dose birds had average plasma protein concentrations that were significantly higher than low dose and control birds. The 3 groups were not significantly different at 7 and 8 weeks PI (Figure 40).

When comparisons were made within groups, the low dose birds had significantly lower average plasma protein concentrations at weeks 0, 1 and 3 PI than they did at weeks 2, 4, 5, 6, 7 and 8 PI.

Average plasma protein concentrations in the high dose group were significantly lower at 1 and 3 weeks PI than they were at 0, 4, 7 and 8 weeks PI. Average concentrations at the latter 4 weeks were significantly lower than those at 2 and 5 weeks PI. Average values at 0 and 3 weeks PI were also significantly lower than those at 4, 5, 6, 7 and 8 weeks PI.

When comparisons were made within the control group, a considerable amount of overlap was detected. Significant differences were not detected among 2, 3, 4, 6 and 7 weeks PI, among 1, 2, 4, 6, 7 and 8 weeks PI and among 1, 2, 5, 6 and 8 weeks PI. Average values at week 0 were significantly lower than those at any other week PI.

Hemoglobin. Statistical analysis of hemoglobin data revealed that all 4 variables in the model statement were highly significant ($p = .0001$).

When comparisons were made by week, high dose birds had significantly lower average hemoglobin values at 4 weeks PI than either low dose or control birds. No differences were significant at other weeks PI.

When comparisons were made within the low dose group, average values at 6, 7 and 8 weeks PI were significantly lower than the average at week 0. Average values at week 0 were significantly lower than those at 3 and 4 weeks PI. Average values at 3 and 4 weeks PI were significantly lower than those at 1, 2 and 5 weeks PI (Figure 41).

Comparisons within the high dose group had considerable overlap. Average values at 4, 6, 7 and 8 weeks PI were significantly lower than those at 1, 3 and 5 weeks PI. Average values at 0, 3 and 7 weeks PI were significantly lower than those at 1, 2 and 5 weeks PI. Significant differences were not detected among 0, 3 and 7

weeks PI, among 0, 3 and 5 weeks PI and among 1, 2 and 5 weeks PI.

Average hemoglobin values within the control group were significantly lower at 6, 7 and 8 weeks PI than they were at week 0. Average values at week 0 were significantly lower than those at 3, 4 and 5 weeks PI. Average values at 3, 4 and 5 weeks PI were significantly lower than those at 1 and 2 weeks PI.

Experiment 2 - Exoerythrocytic Development

Three days. At 3 days post-infection, sections of skeletal muscle from the inoculated poult had a few focal areas of perivascular monocytic infiltrate. Some muscle fibers appeared disrupted, with granular sarcoplasm and scattered islands of pale, disorganized myoglobin (Figure 53). A single, uninucleate parasite with blue-gray granular cytoplasm was detected within a capillary (Figure 42). The organism was 3 μ m in diameter and present in only 1 of a series of 4 μ m serial sections. Other tissues in the infected and control bird were unremarkable.

Five days. By 5 days post-infection, infected poults developed severe lameness in both legs. All had difficulty standing and moving about their cage, while control birds remained active. Skeletal muscle from an infected bird had focal areas of necrosis that involved many

adjacent muscle fibers. The fibers were swollen, pale and hyaline and often had disrupted sarcoplasm (Figure 54). Individual swollen, rounded and hyaline muscle fibers were scattered randomly throughout healthy tissue. A few focal areas of perivascular monocytic infiltrate were present.

Schizonts measuring from 12 to 20 μ m in diameter were present both within and between muscle fibers (Figures 43, 44). The smaller, more immature parasites contained dark-staining, granular cytoplasm (Figure 43). The larger, more mature forms were packed with dark-staining, elongate zoites that were bent and twisted around each other (Figure 44). None of the parasites had an associated host reaction.

Sections of heart from the infected bird had focal aggregates of mononuclear cells scattered randomly throughout the tissue. Hepatocellular atrophy and necrosis were evident in sections of liver. The spleen was enlarged and contained numerous erythropoietic cells in the vascular sinuses. Other tissues from the infected and control bird were unremarkable.

Eight days. By 8 days post-infection, infected birds showed dramatic signs of improvement and were back on their feet. Sections of skeletal muscle from 1 infected bird had numerous focal areas of necrotic muscle fibers, infiltrated

by macrophages and giant cells. Regenerating muscle fibers were common (Figure 55).

Small schizonts with blue-gray cytoplasm and numerous, irregularly shaped black nuclei were present in capillaries. The schizonts were sausage shaped, with an irregular outline and ranged from 5 to 8 μ m in diameter up to 28 μ m in length (Figures 45, 46). Most schizonts extended only as far as 2 to 3, 4 μ m serial sections. Some schizonts were found adjacent to or within the muscle lesions, although most showed no evidence of an associated host response.

Hepatocellular atrophy and necrosis were evident in sections of liver from the infected bird. Numerous large, 10 - 20 μ m, intracellular vacuoles were scattered throughout the tissue. The spleen was enlarged, but otherwise unremarkable. Other tissues from the infected and control bird were unremarkable.

Eleven days. At 11 days post-infection, infected birds continued to improve. Regenerating muscle fibers, often surrounded by a monocytic infiltrate, were common in sections of skeletal muscle from 1 infected bird. Necrotic areas containing giant cells and macrophages were rare. Perivascular nodular infiltrates composed of monocytes and some heterophils were present. Other tissues from the infected and control bird were unremarkable.

Schizonts were not detected in skeletal muscle or other tissues in the infected bird.

Fourteen days. By 14 days post-infection, infected birds were smaller than controls, but were otherwise indistinguishable. Regenerated muscle fibers were common in sections of skeletal muscle from 1 infected bird. The regenerated areas occasionally contained remnants of necrotic fibers that were surrounded by a monocytic infiltrate. Perivascular, nodular infiltrates composed of monocytes were present.

Schizonts ranged from 20 to 32 μ m in diameter and developed both within and between muscle fibers (Figures 47, 48, 49). They were surrounded by a thick, hyaline wall and were packed with dark blue, granular cytomeres that ranged from 2 to 3 μ m in diameter. Schizonts were elongate and extended as far as 90 μ m along the long axis of the muscle fibers. Several schizonts were densely packed with dark-staining granules (Figure 47). Others were surrounded by macrophages (Figure 49).

Sections of heart from the infected bird had focal areas of mononuclear infiltrates. Other tissues from the infected and the control bird were unremarkable.

Seventeen days. At 17 days post-infection, pectoral muscle from the last infected bird contained a few

areas of diffuse, white streaks, several mm in length, embedded deeply in the tissue. These were less distinctive than the white fascia that separated muscle bundles. Fully mature megaloschizonts containing numerous, densely packed, spherical zoites and several large, central vacuoles were present in sections of the tissue (Figure 50, 51). Immature forms resembling 14-day-old schizonts were also present. The megaloschizonts were surrounded by a thick hyaline wall and ranged from 30 to 113 μ m in diameter. They extended as far as 465 μ m along the long axis of muscle fibers. Fibers adjacent to the megaloschizonts were swollen, pale and hyaline (Figure 50). Several layers of connective tissue, infiltrated with macrophages, surrounded some schizonts. A large degenerating megaloschizont, filled with amorphous, gray material containing irregularly shaped red masses, was present in 1 section. The megaloschizont was surrounded by giant cells and an outer layer of connective tissue (Figure 56). Ruptured, partially empty megaloschizonts that contained small numbers of scattered zoites were also present (Figure 52). Each zoite contained a small mass of chromatin and a large vacuole. Giemsa-stained erythrocytes from the same bird contained young gametocytes that were morphologically indistinguishable from the exoerythrocytic zoites.

Sections of heart from the infected bird had focal areas of monocytic infiltrate. A single megaloschizont without an associated host response was present. Other tissues from the infected and control birds were unremarkable.

Exoerythrocytic Development in Natural Infections

In November, 1970, a male Wild Turkey was captured near Lake Apopka, Florida. The bird died soon after capture, was frozen and then necropsied several weeks later by K.P.C. Nair and D.J. Forrester (pers. comm.). At necropsy, scattered whitish cysts, the size of millet seeds, were noticed on the pectoral muscles. Histological examination of the tissue revealed occasional spherical megaloschizonts from 200 - 400 μ m in diameter. The megaloschizonts were surrounded by a thick hyaline wall (Figures 57, 58). Some contained disorganized masses of dark staining material (Figure 57). Others held numerous spherical merozoites (Figure 58). Muscle fibers surrounding some of the megaloschizonts were pale, swollen and had hyaline cytoplasm. Some fibers contained small, dark-staining granules (Figure 58).

Other infections with the organism were not detected in many subsequent necropsies of Wild Turkeys from northern and southern Florida (Forrester, pers. comm.).

Figure 21. Formalized portion of pectoral muscle from a high dose bird that died spontaneously at 19 days post-infection. Megaloschizonts appear as numerous white streaks (arrows) scattered throughout the tissue. The dark flecks and discolored areas are hemorrhagic cysts. Bar = 1 cm.

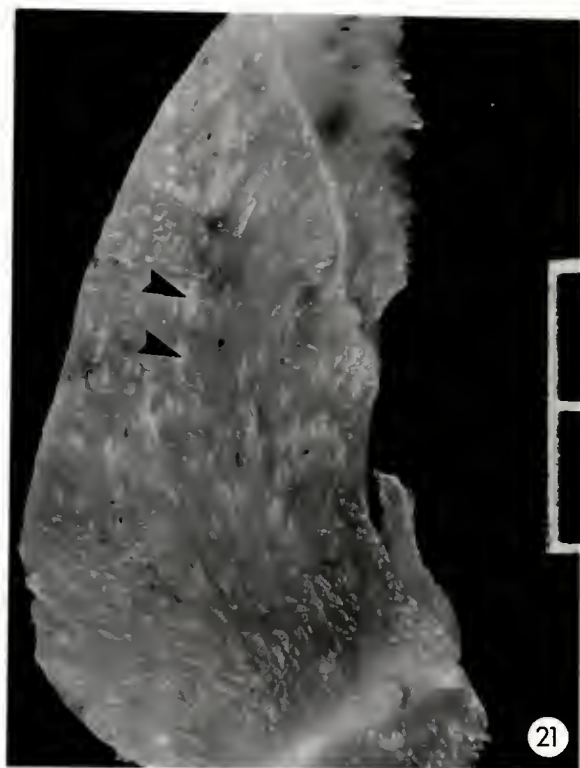


Figure 22. An intact megaloschizont from the pectoral muscle of a high dose bird that died spontaneously at 19 days post-infection. Node-like constrictions (arrows) occur along its length. Hematoxylin and eosin. Bar = 100um.

Figure 23. An intact megaloschizont from pectoral muscle of a high dose bird that died spontaneously at 19 days post-infection. The megaloschizont is surrounded by a thick, hyaline wall (arrows). The interior is packed with minute merozoites. Cytomeres are not evident. The megaloschizont is surrounded by a mixed inflammatory infiltrate composed of giant cells (double arrow), macrophages, heterophils and red blood cells. Hematoxylin and eosin. Bar = 50 um.

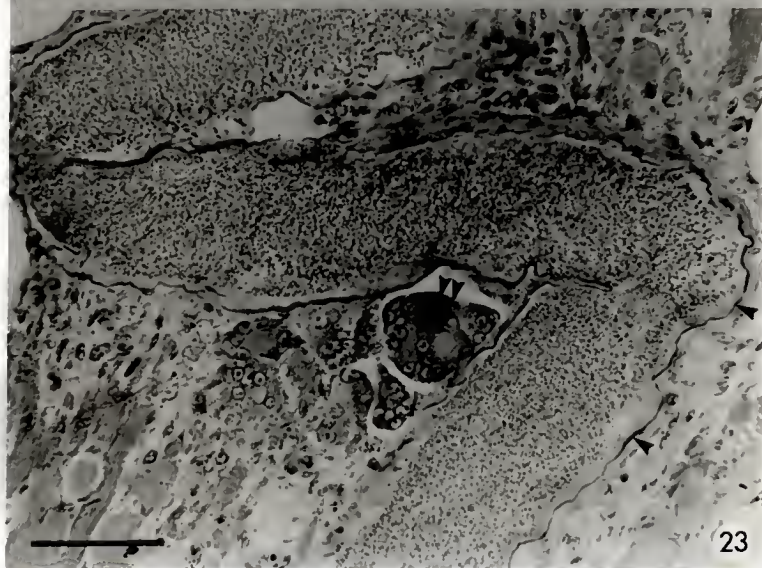


Figure 24. A degenerating megaloschizont from pectoral muscle of a high dose bird that died spontaneously at 19 days post-infection. Hyaline, necrotic muscle fibers (large arrow) and a mononuclear infiltrate (double arrow) surround the schizont. Hematoxylin and eosin. Bar = 50 um.

Figure 25. A degenerating megaloschizont from pectoral muscle of a high dose bird that died spontaneously at 19 days post-infection. The cyst contains numerous mononuclear cells (arrow) and a fibrinous exudate. Giant cells (double arrow) are adjacent to 1 side of the cyst. Hematoxylin and eosin. Bar = 50 um.

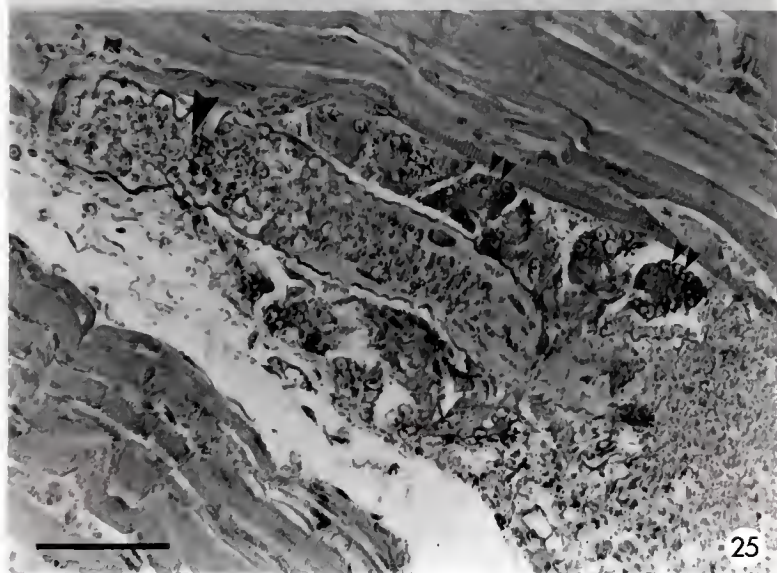
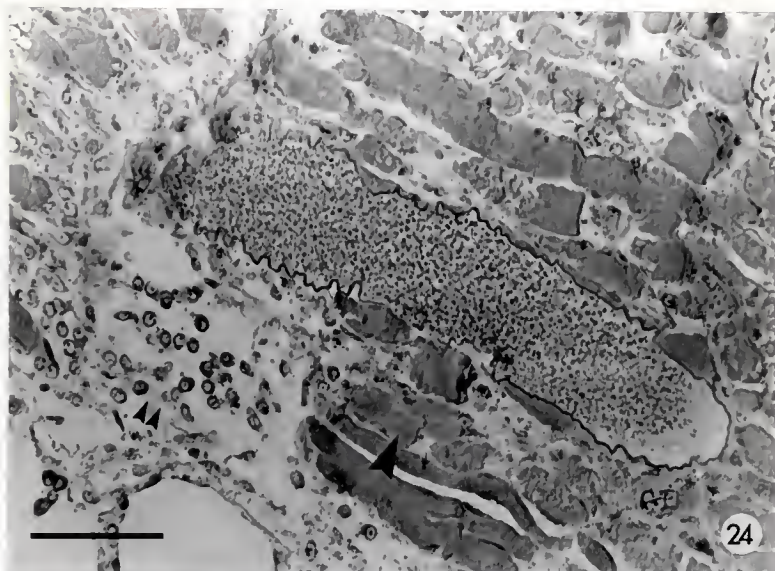


Figure 26. An intact megaloschizont from pectoral muscle of a high dose bird that died spontaneously at 22 days post-infection. The megaloschizont is surrounded by dark-staining areas of calcification. Parallel lines of dark-staining granules are present in 1 muscle fiber adjacent to the schizont (arrow). Hematoxylin and eosin. Bar = 50 um.

Figure 27. A serial section of the megaloschizont illustrated in Figure 26. The section is stained with von Kossa's stain for calcium. There is a close correspondence between the dark-staining deposits in Figure 26 and the dark-staining calcium deposits in Figure 27. Bar = 50 um.

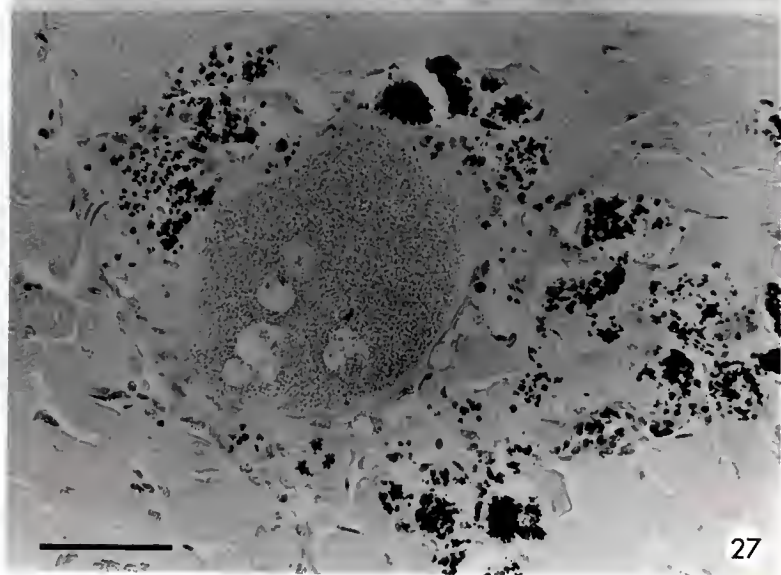
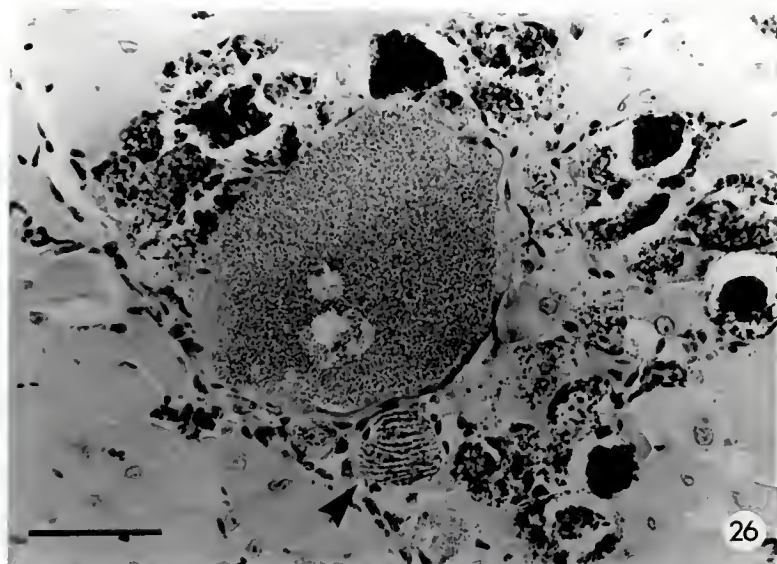


Figure 28. A degenerating megaloschizont from pectoral muscle of a high dose bird that died spontaneously at 22 days post-infection. The hyaline cyst wall (arrow) and a mixed inflammatory infiltrate composed primarily of red blood cells is all that remains of the schizont. Hematoxylin and eosin. Bar = 50 um.

Figure 29. A venule blocked by a thrombus in pectoral muscle of a high dose bird that died spontaneously at 22 days post-infection. The thrombus is surrounded by a giant cell (arrow). Hematoxylin and eosin. Bar = 50 um.

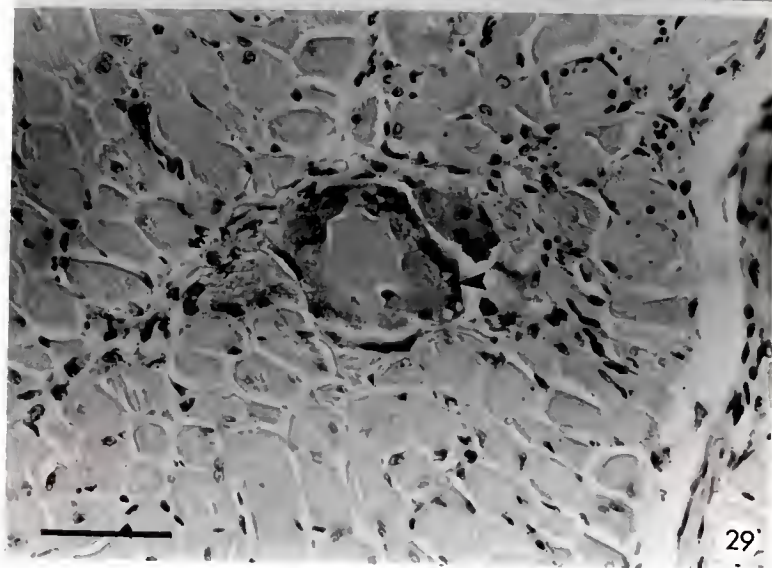
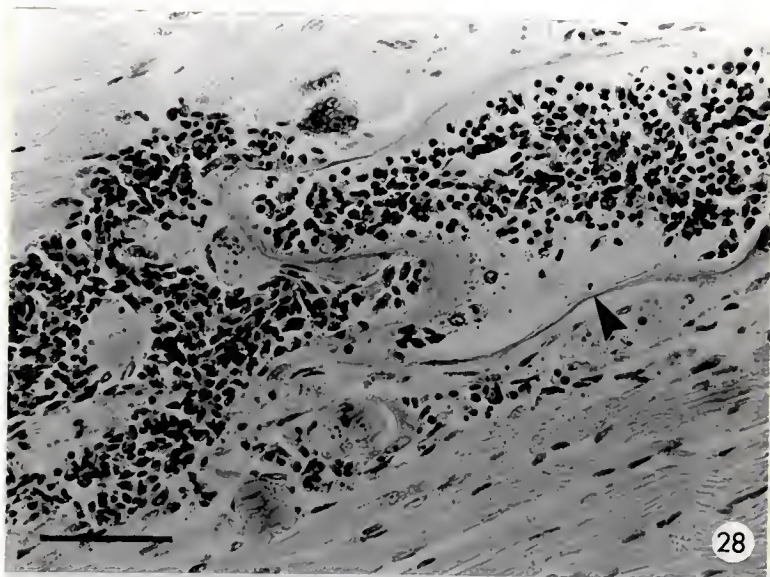


Figure 30. A spleen section from a high dose bird that died spontaneously at 22 days post-infection. Numerous masses of dark pigment are scattered throughout the tissue. Hematoxylin and eosin. Bar = 20 μ m.

Figure 31. A schizont in the spleen of a high dose bird that died spontaneously at 22 days post-infection. The host cell has a pycnotic nucleus (arrow). The schizont is much smaller than megaloschizonts from skeletal muscle in the same bird and lacks a thick, hyaline wall. Hematoxylin and eosin. Bar = 10 μ m.

Figure 32. Schizonts in the spleen of a high dose bird that died spontaneously at 22 days post-infection. One schizont contains elongate zoites (large arrow). Free merozoites (small arrow) are scattered throughout the tissue. Hematoxylin and eosin. Bar = 10 μ m.

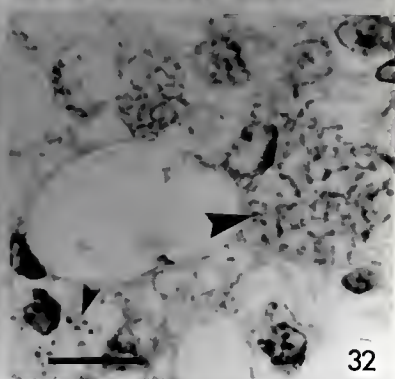
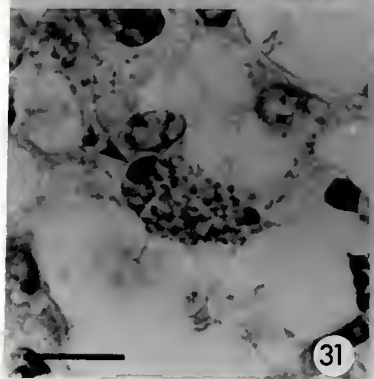
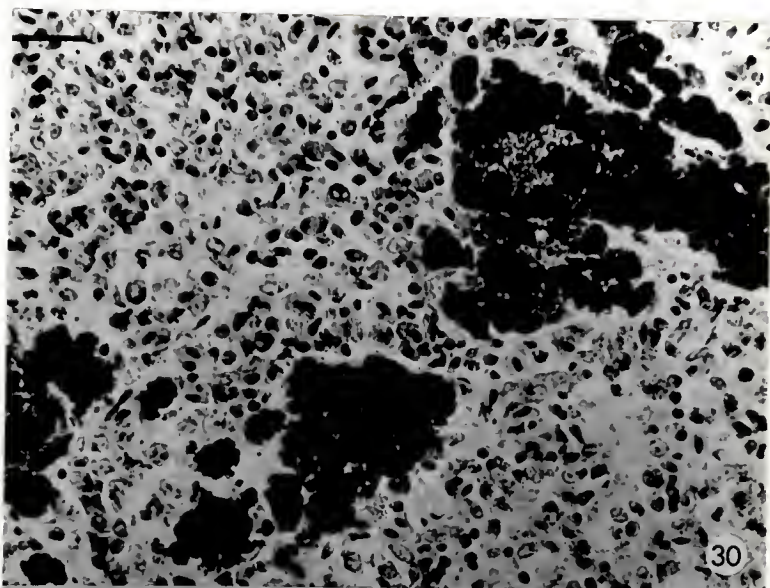


Figure 33. Nodular infiltrate from pectoral muscle of a high dose bird that was killed at 8 weeks post-infection. The infiltrate is composed primarily of mononuclear cells and surrounds some dark-staining areas of calcification (arrow). Hematoxylin and eosin. Bar = 50 um.

Figure 34. A thrombus, composed of fibrinous material, adjacent to the remnants of a degenerating megaloschizont (arrow). The section is from pectoral muscle of a high dose bird that was killed at 8 weeks post-infection. Hematoxylin and eosin. Bar = 20 um.

Figure 35. A mass of degenerating muscle fibers, infiltrated with macrophages and heterophils. Section is from pectoral muscle of a high dose bird that was killed at 8 weeks post-infection. Hematoxylin and eosin. Bar = 20 um.

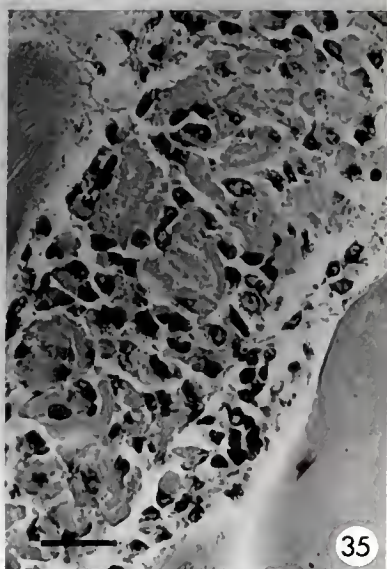
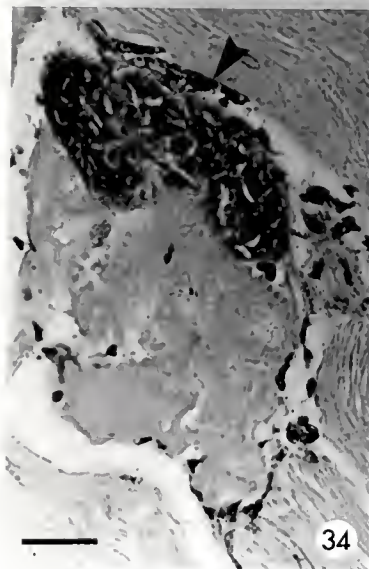
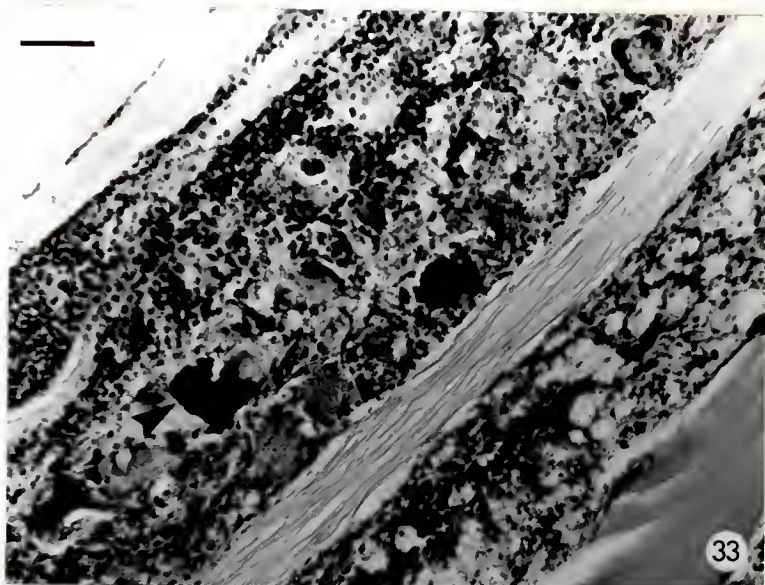


Figure 36. Average parasitemia for high dose birds (O.....O), low dose birds (▲---▲) and control birds (●---●). The crisis occurred on the day when the peak parasitemia was reached.

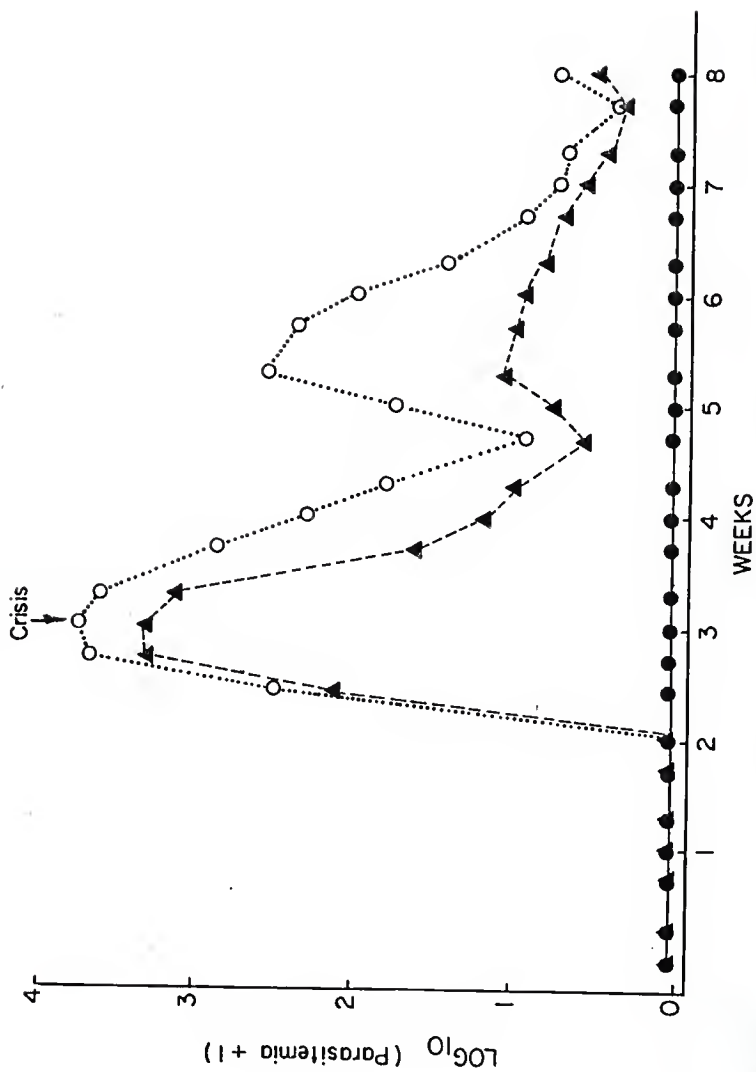


Figure 37. Average weights of high dose birds (O.....O), low dose birds (▲---▲) and control birds (●---●). Statistically significant differences among groups for each week of the study are indicated by the boxed points ($p < 0.05$).

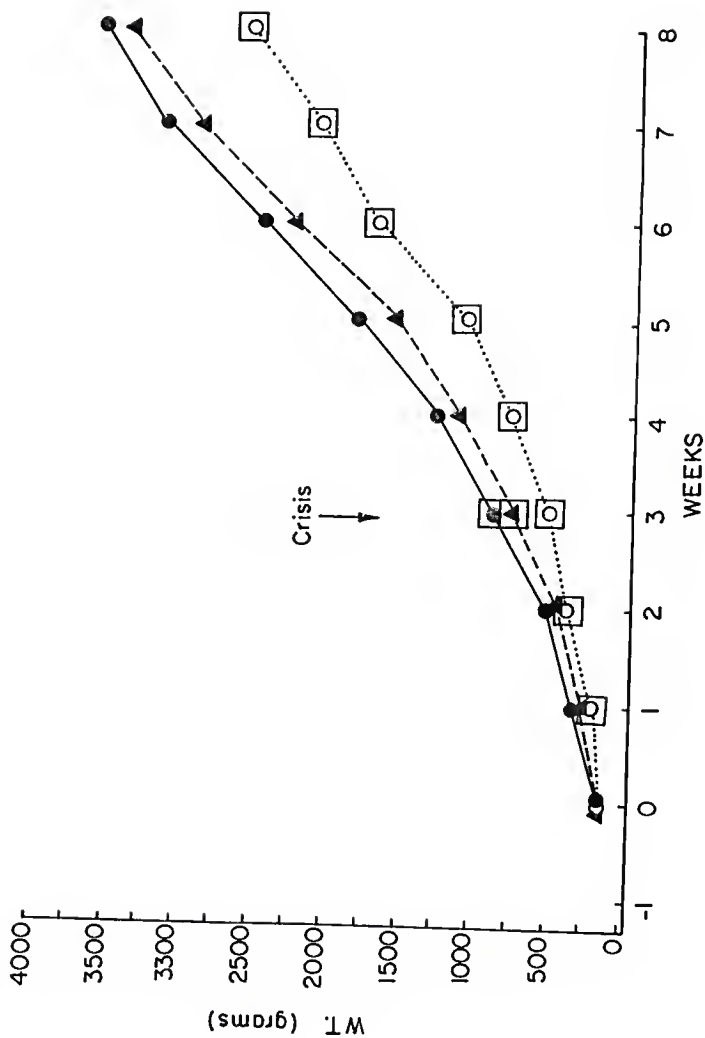


Figure 38. Average tarsometatarsal lengths of high dose birds (O.....O), low dose birds (▲---▲) and control birds (●---●). Statistically significant differences among groups for each week of the study are indicated by the boxed points ($p < 0.05$).

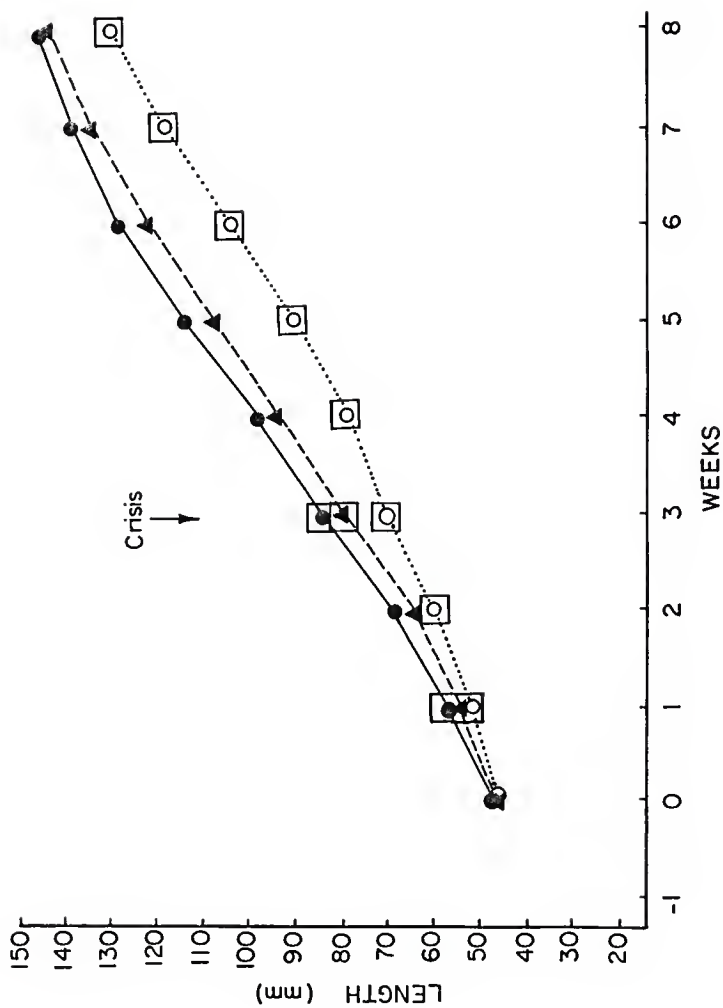


Figure 39. Average hematocrits for high dose birds (O.....O), low dose birds (▲---▲) and control birds (●---●). There were no statistically significant differences among groups when comparisons were made by week ($p < 0.05$).

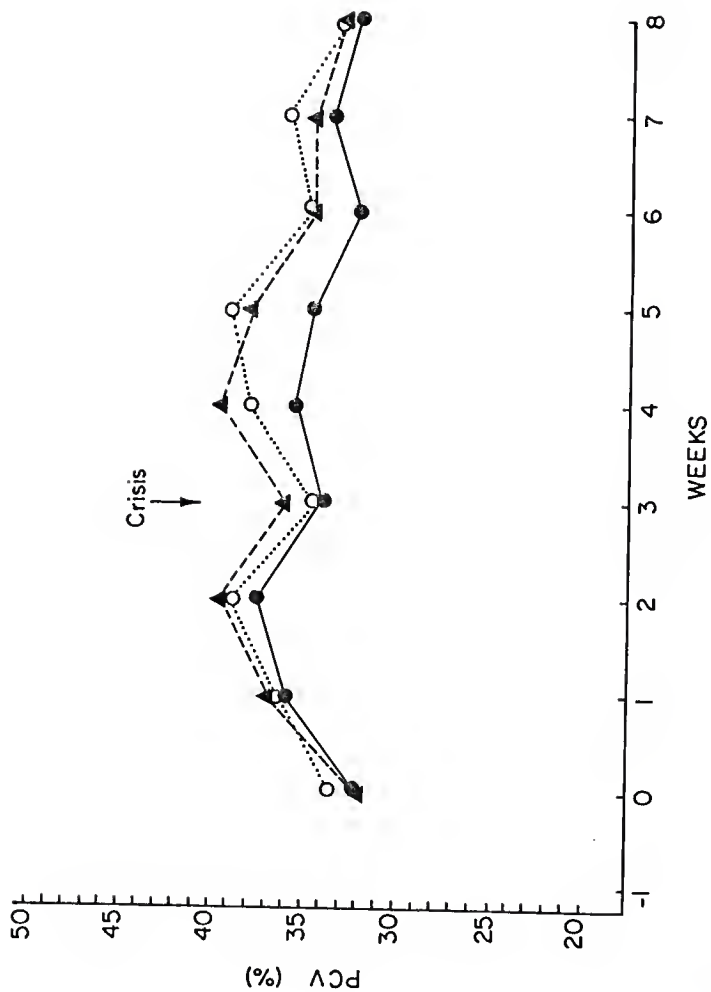


Figure 40. Average plasma protein concentrations for high dose birds (O....O), low dose birds (▲---▲) and control birds (●---●). Statistically significant differences among groups for each week of the study are indicated by the boxed points (p 0.05).

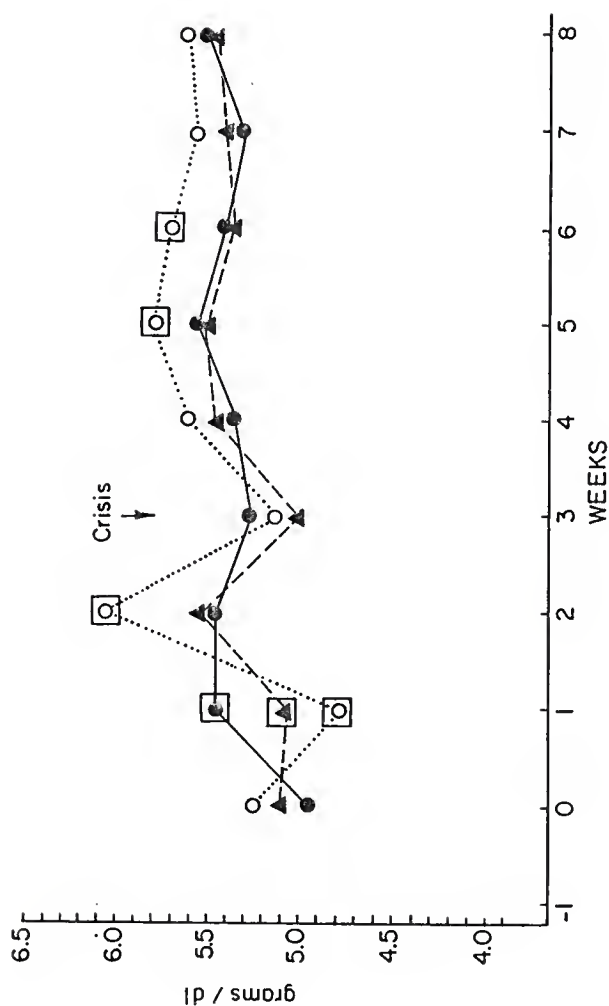
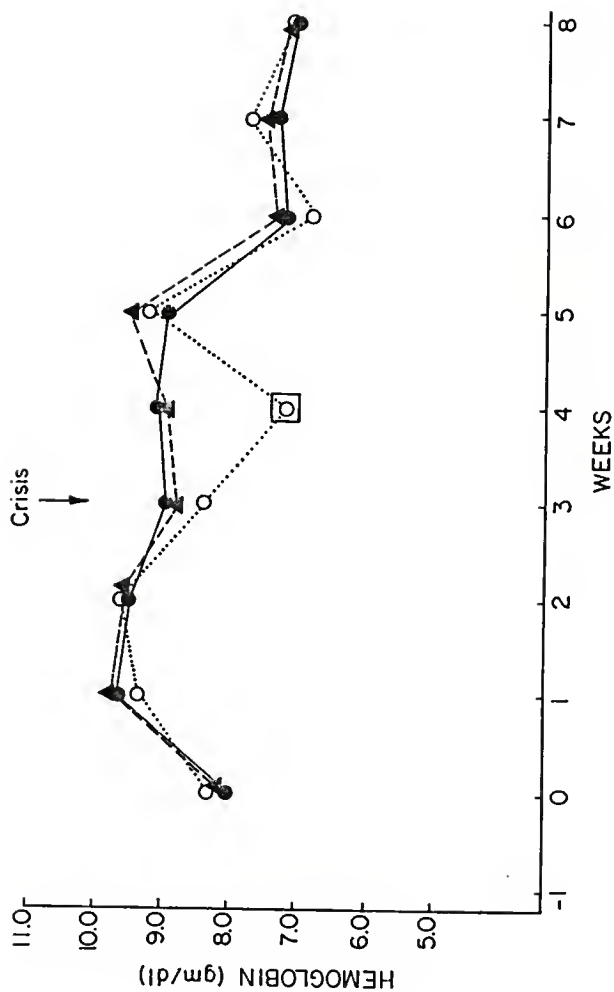


Figure 41. Average hemoglobin concentrations for high dose birds (O.....O), low dose birds (▲---▲) and control birds (●---●). Statistically significant differences among groups for each week of the study are indicated by the boxed points ($p < 0.05$).



- Figure 42. Three-day-old schizont (arrow) from pectoral muscle of an experimentally infected turkey. The schizont is within a capillary. Hematoxylin and eosin. Bar = 10 μ m.
- Figure 43. Five-day-old schizont from pectoral muscle of an experimentally infected turkey. The schizont is located between muscle fibers and is packed with dark granules. Hematoxylin and eosin. Bar = 10 μ m.
- Figure 44. Five-day-old schizont from pectoral muscle of an experimentally infected turkey. The schizont is located within a muscle bundle and is packed with elongate zoites. Hematoxylin and eosin. Bar = 10 μ m.
- Figure 45. Eight-day-old megaloschizont (arrow) from pectoral muscle of an experimentally infected turkey. The megaloschizont is located within a capillary and contains several dark-staining nuclei. Hematoxylin and eosin. Bar = 10 μ m.
- Figure 46. Eight-day-old megaloschizont from pectoral muscle of an experimentally infected turkey. A giant cell (double arrow) is adjacent to the megaloschizont (arrow). Hematoxylin and eosin. Bar = 10 μ m.
- Figure 47. Fourteen-day-old megaloschizont from pectoral muscle of an experimentally infected turkey. The megaloschizont is located within a muscle fiber and is surrounded by a thick, hyaline wall (arrow). Hematoxylin and eosin. Bar = 10 μ m.

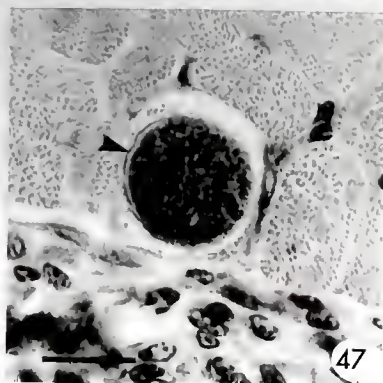
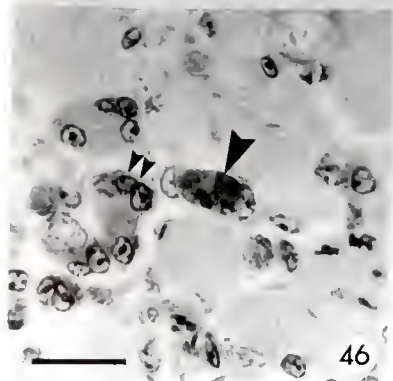
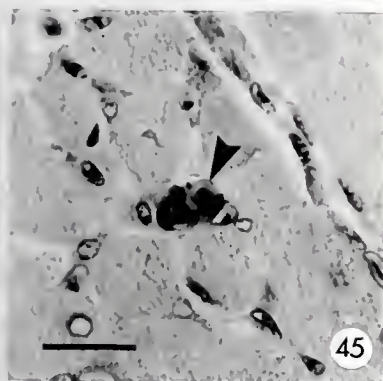
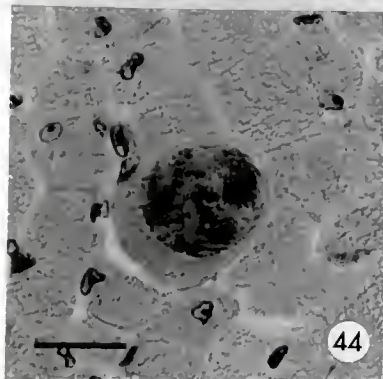
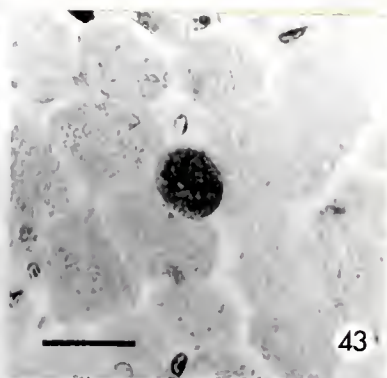
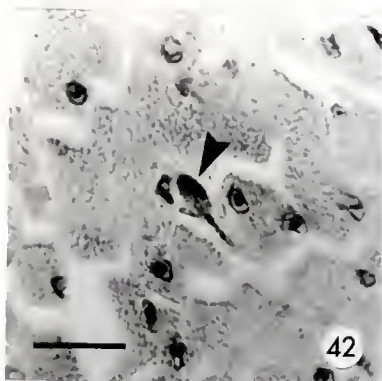


Figure 48. Fourteen-day-old megaloschizont from pectoral muscle of an experimentally infected turkey. The megaloschizont contains dark, spherical cytomeres and is surrounded by a thick, hyaline wall (arrow). Hematoxylin and eosin. Bar = 10 um.

Figure 49. Fourteen-day-old megaloschizont from pectoral muscle of an experimentally infected turkey. The megaloschizont contains spherical, multinucleated cytomeres and is surrounded by a hyaline wall (double arrow) and a mononuclear infiltrate. A swollen muscle fiber with disrupted, hyaline cytoplasm is near the megaloschizont (arrow). Hematoxylin and eosin. Bar = 10 um.

Figure 50. Seventeen-day-old megaloschizont from pectoral muscle of an experimentally infected turkey. The megaloschizont is surrounded by several layers of connective tissue (CT) and contains vacuolated areas (V). Swollen, pale and hyaline muscle fibers (arrows) are adjacent to the megaloschizont. Hematoxylin and eosin. Bar = 50 um.

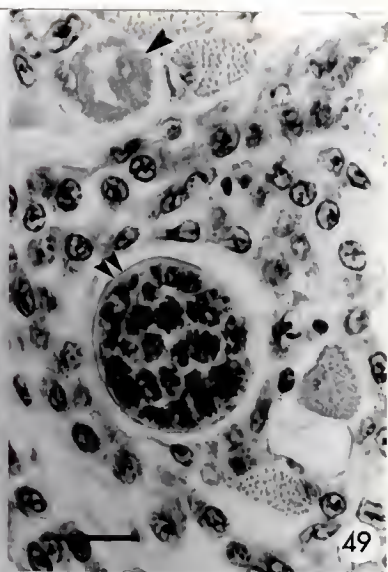
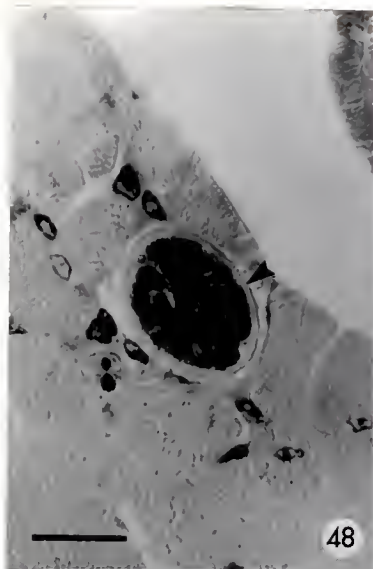
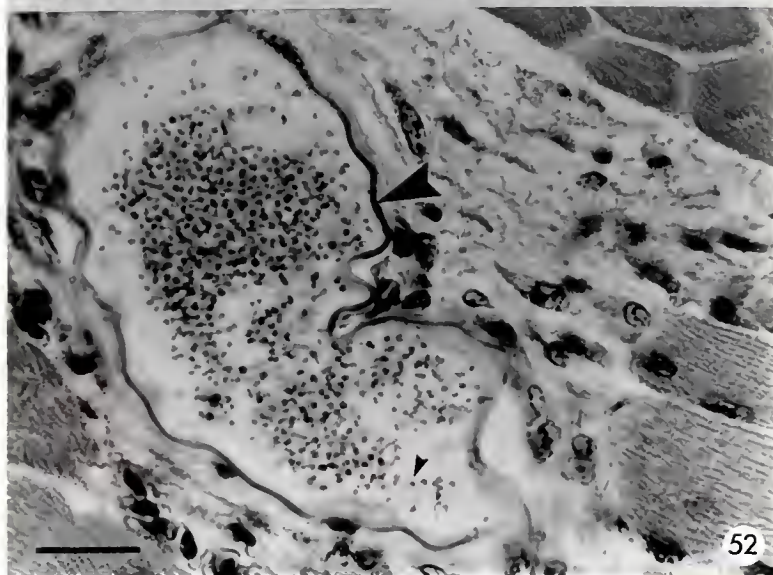
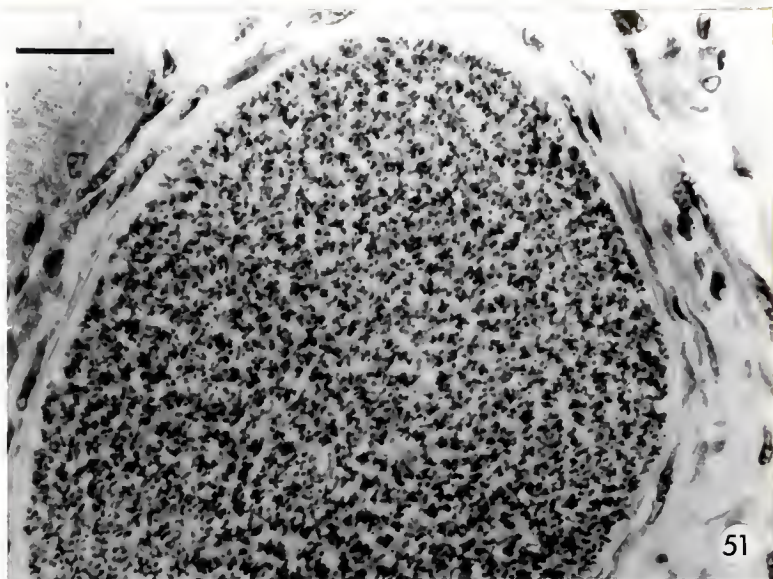


Figure 51. Seventeen-day-old megaloschizont from pectoral muscle of an experimentally infected turkey. The megaloschizont contains numerous elongate and branching cytomeres with budding merozoites. Hematoxylin and eosin. Bar = 10 um.

Figure 52. Seventeen-day-old megaloschizont from pectoral muscle of an experimentally infected turkey. The thick, hyaline outer wall of the megaloschizont (large arrow) has ruptured, liberating the merozoites. The merozoites are spherical and contain a large vacuole (small arrow). Hematoxylin and eosin. Bar = 10 um.



- Figure 53. Disrupted muscle fiber (arrow) from pectoral muscle of a turkey with a 3-day-old experimental infection of Haemoproteus meleagridis. Hematoxylin and eosin. Bar = 10 μ m.
- Figure 54. Swollen, hyaline and disrupted pectoral muscle fibers from a turkey with a 5-day-old experimental infection of Haemoproteus meleagridis. Normal muscle fibers (M) surround the lesion. Hematoxylin and eosin. Bar = 10 μ m.
- Figure 55. Regenerating muscle fibers (arrows) from pectoral muscle of a turkey with an 8-day-old experimental infection of Haemoproteus meleagridis. Hematoxylin and eosin. Bar = 10 μ m.
- Figure 56. Deteriorating 17-day-old megaloschizont (S) from pectoral muscle of an experimentally infected turkey. The megaloschizont (S) is surrounded by giant cells (G) and an outer layer of connective tissue (arrow). Hematoxylin and eosin. Bar = 100 μ m.

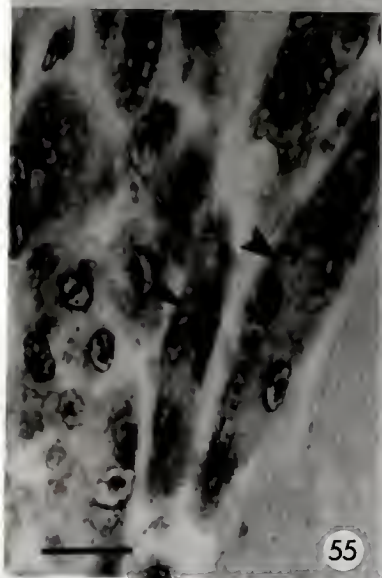
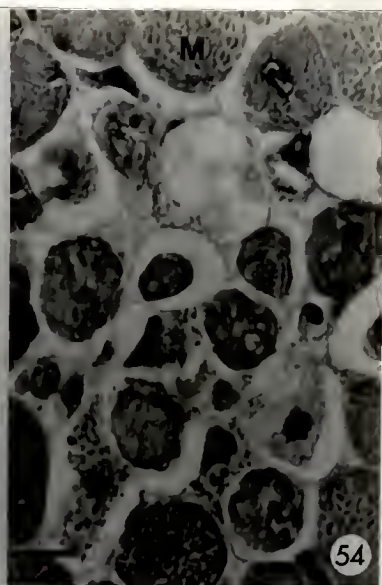
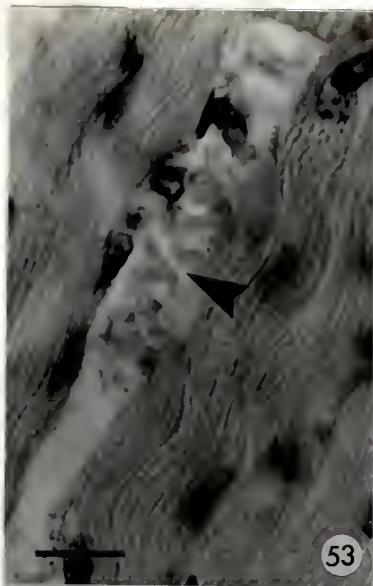
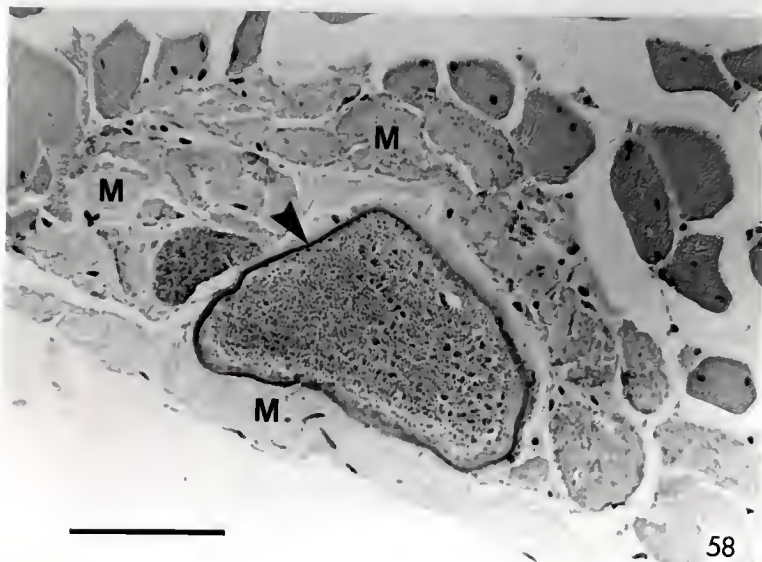
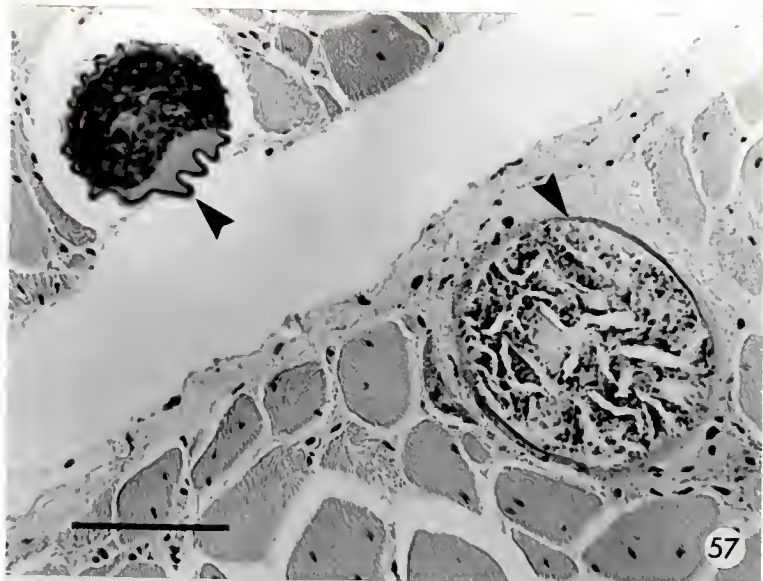


Figure 57. Megaloshizonts from pectoral muscle of a naturally infected Wild Turkey collected near Lake Apopka, Orange County, Florida in November, 1970. Megaloshizonts have a thick, hyaline outer wall (arrows). Hematoxylin and eosin. Bar = 50 μ m.

Figure 58. Megaloshizont from pectoral muscle of a naturally infected Wild Turkey collected near Lake Apopka, Orange County, Florida in November, 1970. Megaloshizont has a thick, hyaline wall (arrow) and is surrounded by pale, swollen and hyaline muscle fibers (M). Hematoxylin and eosin. Bar = 50 μ m.



Host SpecificityParasitemia

Haemoproteus meleagridis was successfully transmitted to 1 of 2 Ring-necked Pheasants, 1 of 2 Chuckars and 2 turkeys that acted as positive controls in the first series of experimental infections. One of the 2 inoculated Guineafowl was found dead 6 days post-infection (DPI). One day prior to death, the bird appeared healthy. At necropsy, all organs appeared normal. Histological sections of liver, lung, heart, kidney, gizzard, pancreas, duodenum, cecum and brain were unremarkable. Skeletal muscle and spleen were not fixed. The surviving inoculated Guineafowl and all negative controls failed to develop patent infections throughout the duration of the experiment.

All infected birds became patent at 17 DPI. The 2 infected turkeys reached an average peak parasitemia of 813 parasites per 10,000 red blood cells at 22 DPI. The parasitemia rapidly fell by 28 DPI to 33 parasites per 10,000 red blood cells and remained at levels less than 15 parasites per 10,000 red blood cells for the duration of the experiment (Figure 59). By contrast, the positive Chuckar reached a lower peak parasitemia of 162 parasites per 10,000 red blood cells at 17 DPI. A rapid drop to 23 parasites per 10,000 red blood cells

occurred by 20 DPI. Gametocytes were cleared from the circulation by 36 DPI. The Ring-necked Pheasant that developed a patent infection proved to be the least susceptible to H. meleagridis. The parasitemia reached a peak of only 22 parasites per 10,000 red blood cells at 20 DPI and was cleared from the peripheral circulation by 26 DPI (Figure 59).

The 2 turkeys inoculated in the second series of experimental infections were the only birds that developed patent infections of H. meleagridis. The Northern Bobwhites, chickens and all negative control birds remained negative throughout the course of the experiment.

Morphometric Analysis

Macrogametocytes. Macrogametocytes from each of the infected host species were morphologically similar (Figures 60, 62, 64). All completely encircled the host cell nucleus and were consistent with descriptions of neotypes of H. meleagridis (Greiner and Forrester, 1980). The average adjusted cell area of macrogametocytes from the turkey was larger than that of macrogametocytes from either the Chuckar or the Ring-necked Pheasant (Table 12). Average values of other adjusted variables were within 1 standard deviation of each other (Table 12).

A discriminant analysis was performed on adjusted variables from a calibration data set derived from 15 macrogametocytes from the Chuckar, 15 from the turkey and 7 from the Ring-necked Pheasant. It derived a function that correctly classified 80% of the discriminant scores from the Chuckar and 80% of those from the turkey (Table 13). It correctly classified only 1 (14.3%) of the 7 scores from the Ring-necked Pheasant. Five of the pheasant scores (71.4%) were incorrectly identified as turkey and 1 (14.3%) failed to meet criteria for classification in any of the 3 categories (Table 13).

A small data set composed of adjusted measurements of 4 macrogametocytes from the turkey, 4 from the Chuckar and 3 from the Ring-necked Pheasant was analyzed to test the validity of the derived function. The derived function correctly classified 100% of the turkey scores, but only 25% of the Chuckar scores and 33% of the pheasant scores - values close to what would be expected by chance alone (Table 14). Three (75%) of the 4 Chuckar scores were incorrectly classified as turkey (Table 14).

Microgametocytes. Microgametocytes from each of the 3 host species were morphologically similar and encircled the host cell nucleus (Figures 61, 63, 65). Average adjusted values of cell area and cell length were smallest for microgametocytes from the Ring-necked Pheasant

(Table 15). However, average cell width was greater in microgametocytes from the pheasant and probably related to the large, lateral displacement of the host cell nucleus (Figure 65). The average adjusted number of pigment granules in microgametocytes from the Chuckar was considerably higher than values from the pheasant and turkey.

A discriminant analysis was performed on adjusted variables from a calibration data set derived from 15 microgametocytes from each host. It correctly identified 73.3% of the discriminant scores from the Chuckar, 60.0% of the scores from the pheasant and 66.7% of the scores from the turkey (Table 16).

The derived function was tested with a smaller data set composed of adjusted measurements of 4 microgametocytes from each of the 3 host species. It correctly identified 3 (75%) Chuckar scores, 3 (75%) turkey scores, but no (0%) pheasant scores. Two pheasant scores were identified as turkey, 1 as Chuckar and 1 failed to meet the criteria for inclusion into any of the 3 categories (Table 17).

Infected host cells - macrogametocytes. Host cells infected with macrogametocytes underwent a number of common changes in each host species (Figures 60, 62, 64). All had greater cell lengths and cell areas than corresponding uninfected cells from the same host species. Infected host

cells from the turkey underwent the greatest enlargement. Cells from all 3 hosts also had atrophied nuclei with smaller lengths, widths and areas than nuclei from unparasitized red blood cells (Table 18). Infected host cells from the Ring-necked Pheasant underwent the greatest nuclear atrophy.

A discriminant analysis was performed on adjusted variables from a calibration data set composed of measurements of 15 Chuckar cells infected with macrogametocytes, 15 infected turkey cells and 7 infected pheasant cells. It correctly identified 73.3% of the Chuckar scores, 66.7% of the turkey scores and none of the pheasant scores. Three (42.9%) of the pheasant scores were identified as Chuckar and 3 (42.9%) were identified as turkey - percentages approximately equal to what would be expected by chance alone (Table 19). One pheasant score failed to meet the criteria for inclusion in any of the 3 categories.

The derived function was tested with a smaller data set composed of adjusted measurements of 4 host cells from the Chuckar and turkey and 3 host cells from the pheasant. Three (75%) of the Chuckar scores were classified as turkey and 2 (66.7%) of the pheasant scores were classified as Chuckar. Two (50%) turkey scores were correctly identified, but the classification was only slightly less than would be expected by chance alone (Table 20).

Infected host cells - microgametocytes. Host cells infected with microgametocytes underwent several common changes in each host species. All had an increase in average cell length, width and area and a corresponding decrease in nucleus length, width and area (Figures 61, 63, 65). A lateral displacement of the host cell nucleus occurred in parasitized red cells from each host. The displacement was greatest in host cells from the Ring-necked Pheasant and least in host cells from the Chuckar (Table 21). Differences in infected host cell morphology were most evident in cells from the pheasant. Infected red blood cells were rounder than those from the other host species and the red blood cell nucleus was often displaced to the outer margin of the host cell.

A discriminant analysis was performed on a calibration data set composed of adjusted measurements of 15 host cells, infected with microgametocytes, from each host species. It correctly classified 60% of the Chuckar scores, 46.7% of the pheasant scores and 60% of the turkey scores (Table 22).

The derived function was tested with a smaller data set composed of adjusted measurements of 4 host cells from each of the 3 host species. Two (50%) of the Chuckar scores were classified as Chuckar, 1 (25%) of the turkey scores was classified as turkey and 4 (100%) of the

pheasant scores were correctly classified as pheasant (Table 23).

Fine Structure

Mature Gametocytes

Microgametocytes and macrogametocytes were bound by a pellicle composed of 3 unit membranes. The innermost membrane was thicker and more osmiophilic than the outer 2 (Figures 66, 67). Other common organelles included a nucleus bounded by 2 unit membranes, mitochondria with tubular cristae and food vacuoles that contained osmiophilic masses of pigment (Figures 66, 67). Both macrogametocytes and microgametocytes had granular nucleoplasm with an electron density similar to the cytoplasm (Figures 66, 67). Macrogametocytes had an electron-dense nucleolus (Figure 67).

Mature macrogametocytes were packed with numerous ribosomes that gave the cytoplasm a granular appearance (Figure 67). By contrast, mature microgametocytes contained fewer ribosomes and had a paler, more amorphous cytoplasm (Figure 66). Rough endoplasmic reticulum and a Golgi apparatus were not observed. However, gametocytes had a network of smooth endoplasmic reticulum that extended throughout the cytoplasm. In

Table 12. Average adjusted measurements of macrogametocytes. Average measurements of each variable are expressed as a percentage of the average uninfected host cell area for that species.

Variable	Chuckar	Pheasant	Turkey
Parasite Length	43.2 (4.29)+	39.9 (1.59)	43.7 (2.47)
Parasite Width	4.4 (1.11)	3.6 (0.82)	4.9 (0.64)
Parasite Area	97.9 (10.8)	104.3 (7.71)	117.2 (9.49)
Nucleus Length	8.8 (1.37)	7.9 (1.08)	7.2 (1.29)
Nucleus Width	4.1 (0.83)	3.3 (0.68)	4.1 (0.59)
Nucleus Area	13.9 (2.36)	11.4 (2.22)	13.3 (2.79)
Pigment*	46.1 (14.4)	53.0 (5.55)	46.9 (6.31)
N =	15	7	15

+ Standard deviation

* Adjusted average of number of pigment granules.

Table 13. Classification summary of a nearest neighbor analysis of a set of calibration data composed of adjusted measurements of macrogametocytes. Discriminant scores were classified with a discriminant function derived from the calibration data set summarized in Table 12.

Species	Classified Into Species				
	Chuckar	Pheasant	Turkey	Other	Total
Chuckar	12 (80.0)+	0 (0.0)	2 (13.3)	1 (5.7)	15 (100)
Pheasant	0 (0.0)	1 (14.3)	5 (71.4)	1 (14.3)	7 (100)
Turkey	2 (13.3)	0 (0.0)	12 (80.0)	1 (6.7)	15 (100)
Total	14 (37.8)	1 (2.7)	19 (51.4)	3 (8.1)	37 (100)
Priors*	0.4054	0.1892	0.4054		

+ Percent of total

* Prior probability of being assigned to that class

Table 14. Classification summary of a nearest neighbor analysis of a set of test data composed of adjusted measurements of macrogametocytes. The discriminant function derived from data in Table 12 was used to classify the discriminant scores.

Species	Classified into Species				Total
	Chuckar	Pheasant	Turkey	Other	
Chuckar	1 (25.0)+	0 (0.0)	3 (75.0)	0 (0.0)	4 (100)
Pheasant	1 (33.3)	1 (33.3)	1 (33.3)	0 (0.0)	3 (100)
Turkey	0 (0.0)	0 (0.0)	4 (100)	0 (0.0)	4 (100)
Total	2 (18.2)	1 (9.1)	8 (72.7)	0 (0.0)	11 (100)
Priors*	0.4054	0.1892	0.4054		

+ Percent of total

* Prior probability of being assigned to that class

Table 15. Average adjusted measurements of microgametocytes. Average measurements of each variable are expressed as a percentage of the average uninfected host cell area.

Variable	Chuckar	Pheasant	Turkey
Parasite Length	41.4 (1.96)+	32.2 (5.65)	37.5 (6.01)
Parasite Width	4.6 (0.67)	6.1 (1.21)	5.8 (0.67)
Parasite Area	103.7 (9.03)	86.1 (8.38)	106.3 (10.6)
Pigment*	39.5 (12.0)	25.3 (5.88)	24.3 (4.72)
N =	15	15	15

+ Standard deviation

* Adjusted average of number of pigment granules.

Table 16. Classification summary of a nearest neighbor analysis of a set of calibration data composed of adjusted measurements of microgametocytes. Discriminant scores were classified with a discriminant function derived from the calibration data set summarized in Table 15.

Species	Classified Into Species				
	Chuckar	Pheasant	Turkey	Other	Total
Chuckar	11 (73.3)+	2 (13.3)	2 (13.3)	0 (0.0)	15 (100)
Pheasant	0 (0.0)	9 (60.0)	5 (33.3)	1 (6.7)	15 (100)
Turkey	3 (20.0)	2 (13.3)	10 (66.7)	0 (0.0)	15 (100)
Total	14 (31.1)	13 (28.9)	17 (37.8)	1 (2.2)	45 (100)
Priors*	0.3333	0.3333	0.3333		

+ Percent of total

* Prior Probability of being assigned to that class

Table 17. Classification summary of a nearest neighbor analysis of a set of test data composed of adjusted measurements of microgametocytes. The discriminant function derived from data in table 15 was used to classify the discriminant scores.

Species	Classified Into Species				Total
	Chuckar	Pheasant	Turkey	Other	
Chuckar	3 (75.0)+	0 (0.0)	1 (25.0)	0 (0.0)	4 (100)
Pheasant	1 (25.0)	0 (0.0)	2 (50.0)	1 (25.0)	4 (100)
Turkey	1 (25.0)	0 (0.0)	3 (75.0)	0 (0.0)	4 (100)
Total	5 (41.7)	0 (0.0)	6 (50.0)	1 (8.3)	12 (100)
Priors*	0.3333	0.3333	0.3333		

+ Percent of total

* Prior probability of being assigned to that class

Table 18. Average adjusted measurements of host cells infected with macrogametocytes. Each variable was divided by the average value of the same variable from uninfected cells of the same species.

Variable	Chuckar	Pheasant	Turkey
Cell Length	1.12 (0.08)+	1.10 (0.05)	1.09 (0.08)
Cell Width	1.02 (0.08)	1.00 (0.07)	1.20 (0.08)
Cell Area	1.15 (0.10)	1.18 (0.08)	1.33 (0.10)
Nucleus Length	0.94 (0.08)	0.76 (0.13)	0.90 (0.10)
Nucleus Width	0.97 (0.09)	0.96 (0.09)	0.97 (0.13)
Nucleus Area	0.95 (0.12)	0.73 (0.11)	0.86 (0.16)
NDR*	0.86 (0.28)	0.93 (0.24)	0.88 (0.16)
N =	15	7	15

+ Standard Deviation

* Nuclear Displacement Ratio (1 = no lateral displacement)
(0 = lateral displacement to the cell margin)

Table 19. Classification summary of a nearest neighbor analysis of a set of calibration data composed of adjusted measurements of host cells infected with macrogametocytes. Discriminant scores were classified with a discriminant function derived from the calibration data set summarized in Table 18.

Species	Classified Into Species				
	Chuckar	Pheasant	Turkey	Other	Total
Chuckar	11 (73.3)+	0 (0.0)	3 (20.0)	1 (6.7)	15 (100)
Pheasant	3 (42.9)	0 (0.0)	3 (42.9)	1 (14.3)	7 (100)
Turkey	3 (20.0)	0 (0.0)	10 (66.7)	2 (13.3)	15 (100)
Total	17 (46.0)	0 (0.0)	16 (43.2)	4 (10.8)	37 (100)
Priors*	0.4054	0.1892	0.4054		

+ Standard Deviation

* Prior probability of being assigned to that class

Table 20. Classification summary of a nearest neighbor analysis of a set of test data composed of adjusted measurements of host cells infected with macrogametocytes. The discriminant function derived from data in Table 18 was used to classify the discriminant scores.

Species	Classified Into Species				
	Chuckar	Pheasant	Turkey	Other	Total
Chuckar	1 (25.0)+	0 (0.0)	3 (75.0)	0 (0.0)	4 (100)
Pheasant	2 (66.7)	0 (0.0)	1 (33.3)	0 (0.0)	3 (100)
Turkey	1 (25.0)	0 (0.0)	2 (50.0)	1 (25.0)	4 (100)
Total	4 (36.4)	0 (0.0)	6 (54.6)	1 (9.1)	11 (100)
Priors*	0.4054	0.1892	0.4054		

+ Percentage of total

* Prior probability of being assigned to that class

Table 21. Average adjusted measurements of host cells infected with microgametocytes. Each variable was divided by the average value of the same variable from uninfected cells of the same species.

Variable	Chuckar	Pheasant	Turkey
Cell Length	1.17 (0.09)+	1.03 (0.07)	1.11 (0.06)
Cell Width	1.02 (0.08)	1.17 (0.08)	1.11 (0.09)
Cell Area	1.18 (0.10)	1.21 (0.08)	1.27 (0.11)
Nucleus Length	0.92 (0.11)	0.82 (0.11)	0.99 (0.11)
Nucleus Width	0.84 (0.11)	0.98 (0.17)	0.95 (0.10)
Nucleus Area	0.77 (0.17)	0.81 (0.15)	0.94 (0.16)
NDR*	0.89 (0.15)	0.28 (0.21)	0.42 (0.26)
N =	15	15	15

+ Standard Deviation

* Nuclear Displacement Ratio (1 = no lateral displacement)
(0 = lateral displacement to cell margin)

Table 22. Classification summary of a nearest neighbor analysis of a set of calibration data composed of adjusted measurements of host cells infected with microgametocytes. Discriminant scores were classified with a discriminant function derived from the calibration data set in Table 21.

Species	Classified Into Species				
	Chuckar	Pheasant	Turkey	Other	Total
Chuckar	9 (60.0)+	0 (0.0)	3 (20.0)	3 (20.0)	15 (100)
Pheasant	0 (0.0)	7 (46.7)	6 (40.0)	2 (13.3)	15 (100)
Turkey	3 (20.0)	2 (13.3)	9 (60.0)	1 (6.7)	15 (100)
Total	12 (26.7)	9 (20.0)	18 (40.0)	6 (13.3)	45 (100)
Priors*	0.3333	0.3333	0.3333		

+ Standard Deviation

* Prior probability of being assigned to that class

Table 23. Classification summary of a nearest neighbor analysis of a set of test data composed of adjusted measurements of host cells infected with microgametocytes. The discriminant function derived from data in Table 21 was used to classify the discriminant scores.

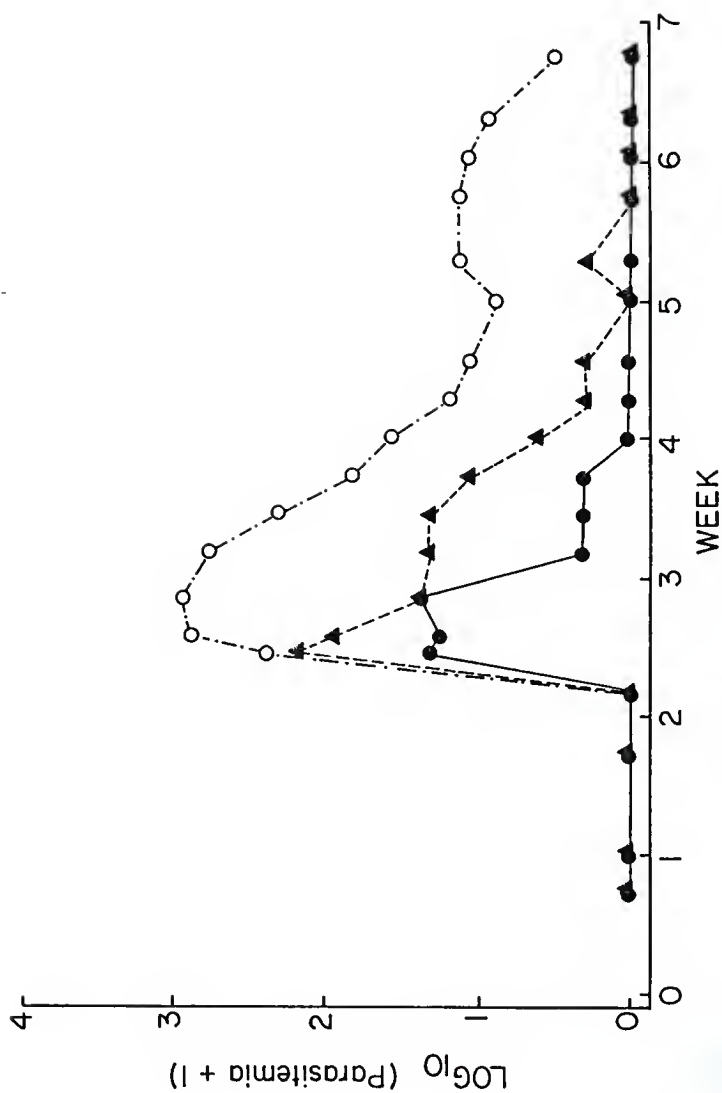
Species	Classified Into Species				Total
	Chuckar	Pheasant	Turkey	Other	
Chuckar	2 (50.0)+	1 (25.0)	0 (0.0)	1 (25.0)	4 (100)
Pheasant	0 (0.0)	4 (100)	0 (0.0)	0 (0.0)	4 (100)
Turkey	1 (25.0)	1 (25.0)	1 (25.0)	1 (25.0)	4 (100)
Total	3 (25.0)	6 (50.0)	1 (8.3)	2 (16.7)	12 (100)
Priors*	0.3333	0.3333	0.3333		

+ Standard Deviation

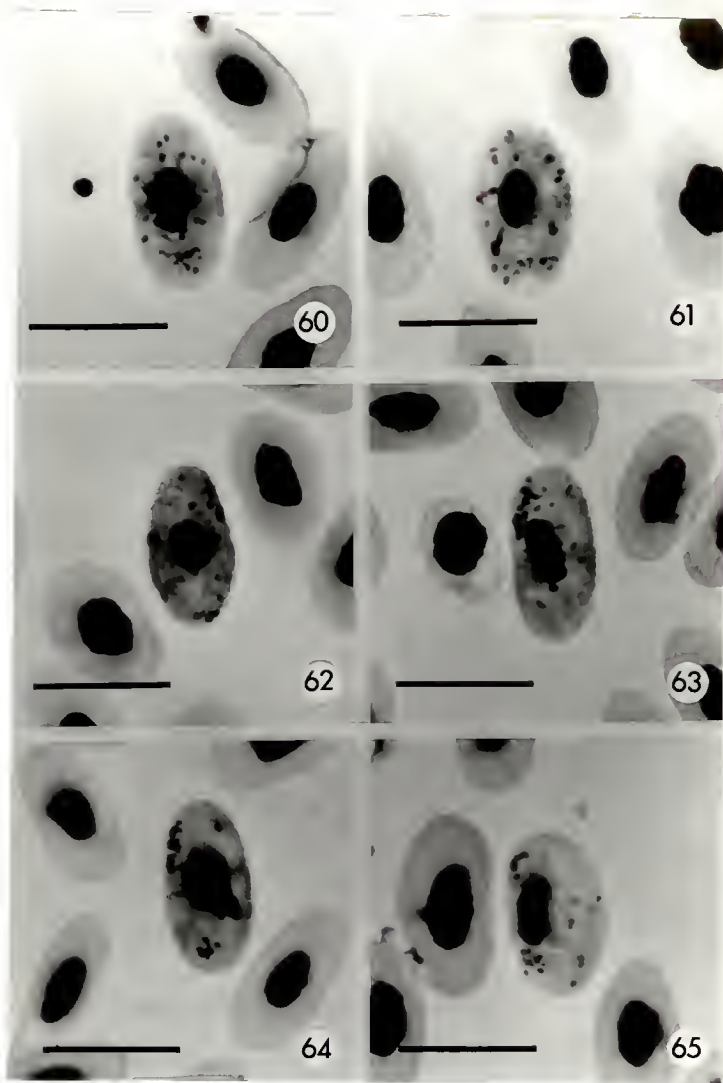
* Prior probability of being assigned to that class

Figure 59.

Parasitemias per 10,000 red blood cells for turkeys, (O---O), the Chuckar (▲---▲) and the Ring-necked Pheasant (●---●) with experimental infections of *Haemoproteus meleagridis*. Values for turkey were calculated as the average parasitemia for the 2 infected birds. All birds received the same number of sporozoites.



- Figure 60. Macrogametocyte of Haemoproteus meleagridis from an experimentally infected turkey. Giemsa. Bar = 10 um.
- Figure 61. Microgametocyte of Haemoproteus meleagridis from an experimentally infected turkey. Giemsa. Bar = 10 um.
- Figure 62. Macrogametocyte of Haemoproteus meleagridis from an experimentally infected Chuckar. Giemsa. Bar = 10 um.
- Figure 63. Microgametocyte of Haemoproteus meleagridis from an experimentally infected Chuckar. Giemsa. Bar = 10 um.
- Figure 64. Macrogametocyte of Haemoproteus meleagridis from an experimentally infected Ring-necked Pheasant. Giemsa. Bar = 10 um.
- Figure 65. Microgametocyte of Haemoproteus meleagridis from an experimentally infected Ring-necked Pheasant. Giemsa. Bar = 10 um.



macrogametocytes, the endoplasmic reticulum contained a moderately dense, amorphous material (Figure 68). The endoplasmic reticulum was occasionally continuous with the innermost, osmiophilic layer of the pellicle (Figure 68). Macrogametocytes also contained membrane-bound, osmiophilic bodies (Figure 67).

Gametocytes contained a single cytostome, surrounded by 2 electron dense rings (Figure 69). Food vacuoles containing host cell cytoplasm and limited by the 2 outer membranes of the pellicle formed at the inner surface of the cytostome, between the 2 electron dense rings. Other food vacuoles in the macrogametocyte were bound by a single unit membrane and contained osmiophilic masses of pigment (Figure 69).

Gametogenesis

In the earliest stages of gametogenesis, gametocytes rounded-up within their host cells (Figures 70, 71, 74). The outer unit membrane of the 3-layered pellicle separated from the parasite and formed membranous vesicles, coils and whorls or remained free as discrete fragments in the red blood cell cytoplasm (Figures 70, 72, 73). Dense granules, approximately the same size and electron density as free ribosomes, occasionally lined the inner side of the membrane in a single layer (Figure 73). The thickened, osmiophilic inner layer of the pellicle of

microgametocytes developed breaks and discontinuities throughout its surface so that the middle layer of the pellicle became the new, outer limiting membrane of the parasite (Figures 71, 72). By contrast, the inner layer of macrogametocytes remained intact (Figure 74).

Macrogametocyte nuclei were elongate and extended across the diameter of the parasites. A prominent nucleolus was present at 1 pole. A kinetic center composed of a mass of amorphous, electron-dense material was adjacent to the nuclear envelope at the other pole (Figure 74).

Within 3 minutes after gametogenesis began, macrogametocytes and microgametocytes were free of their host cells. Both were bound by the middle layer of the original 3-layered pellicle. The pale, electron lucent remnants of ruptured host cell nuclei as well as remnants of host cell membranes were often adjacent to or surrounded free gametocytes (Figure 77). Both maturing macrogametes and exflagellating microgametocytes contained a single cytostome (Figures 76, 78).

Maturing macrogametes were packed densely with ribosomes and contained numerous mitochondria and an extensive network of smooth endoplasmic reticulum (Figure 75). They contained an elongate nucleus with a prominent nucleolus that extended across the center of the macrogametocyte (Figure 75).

Microgametocytes contained a large, diffuse nucleus (Figure 77). Dense aggregates of electron dense material with embedded microtubules were occasionally located adjacent to the nuclear envelope (Figure 77). Axonemes in various stages of assembly were scattered in the cytoplasm of exflagellating microgametocytes and often extended from the nucleus to the outer limiting membrane of the parasite. In cross section, they consisted of 9 peripheral doublets of microtubules that surrounded 2 central tubules (Figures 77, 78). In longitudinal section, the central tubules had regular striations along their length (Figure 79). As development progressed, axonemes budded from the outer surface of the parasite, between interrupted portions of the osmiophilic inner layer of the pellicle (Figure 80). Portions of the microgametocyte nucleus were occasionally drawn to the base of flagellar buds (Figures 80, 81). Exflagellated microgametes contained a single axoneme and a membrane-bound nucleus (Figure 82).

Oocysts

Three-day-old oocysts. Three-day-old oocysts were subspherical in shape and surrounded by a thick, amorphous wall (Figure 83). Oocysts were located under the basement membrane of the midgut. The 2 structures had the same

electron density and were often indistinguishable in areas where the basement membrane was stretched tightly over the oocyst wall (Figure 83).

The pellicle surrounding the 3-day-old sporoblast was immediately interior to the oocyst wall. It was composed of a single unit membrane, underlaid in many places with dark, osmiophilic thickenings (Figure 83).

Prominent, membrane-bound, lipid-like inclusions were clustered together near the center of 3-day-old oocysts (Figure 83). Surrounding these and scattered throughout the cytoplasm were numerous mitochondria with tubular cristae (Figure 83). Several irregularly shaped nuclei with prominent nucleoli were present around the periphery of the parasites. The nucleoplasm had a density similar to the cytoplasm and was difficult to discern in areas where the nuclear envelope was indistinct (Figure 83). Spindle fibers and kinetic centers were not observed.

Six-day-old oocysts. By 6 days, oocysts ranged in development from immature forms, resembling 3-day-old oocysts, to mature oocysts that had ruptured and released their sporozoites. Oocysts that were more mature than the 3-day-old forms had more space between the sporoblast body and the oocyst wall. Numerous budding sporozoites developed around the periphery of the sporoblast. The buds originated under the osmiophilic

thickenings beneath the pellicle (Figure 84). As the buds became more elongate, apical organelles including polar rings, electron dense rhoptries and subpellicular microtubules differentiated from the sporoblast body (Figure 85). One budding sporozoite had at least 18 subpellicular microtubules in cross section (Figure 85). The pellicle of developing sporozoites consisted of an outer unit membrane that was underlaid by 2 unit membranes in close apposition to one another (Figure 85). Large nuclei were located at the periphery of the sporoblast body, underneath the budding sporozoites. Remnants of the apical complex of the ookinete, consisting of an electron dense canopy and supporting microtubules were present within the sporoblast (Figure 84). Large lipid-like vesicles were clustered near the center of the sporoblast body (Figure 84).

As sporozoites completed budding, the sporoblast body became progressively smaller, leaving a residual mass composed of large, lipid-like vesicles and amorphous cytoplasm (Figure 86). At maturity, oocysts ruptured, liberating sporozoites into the haemocoel of the insect. Ruptured oocysts contained the degenerating remnants of the residual body (Figure 87).

Megaloschizonts

Mature, 19-day-old megaloschizonts were extracellular and surrounded by a thick, laminated cyst wall composed of electron dense, amorphous material (Figure 88). Host tissue adjacent to megaloschizonts was necrotic and infiltrated with phagocytic cells (Figure 88). The region between healthy muscle tissue and megaloschizonts contained large quantities of amorphous, granular material with the same electron density as the cyst wall (Figure 88), membranous vesicles, free mitochondria with swollen and ruptured cristae and scattered fragments of myofibrils. Many mitochondria were opaque and electron dense. The amorphous, granular material exterior to the megaloschizonts appeared to be deposited in layers onto the outer surface of the cyst wall (Figure 88). Phagocytic cells adjacent to ruptured megaloschizonts were often packed with merozoites.

Megaloschizonts contained merozoites that developed as buds from discrete cytomeres (Figure 89). The merozoites were bound by a single unit membrane that was underlaid by an interrupted intra-membranous layer composed of 2 unit membranes in close apposition to one another (Figure 89). Merozoites had 3 anterior polar rings, a pair of electron-dense rhoptries, micronemes, a nucleus bound by 2 unit membranes and a single mitochondrion with tubular cristae (Figures 89, 90, 91). All merozoites also had a

large, electron lucent, membrane-bound vacuole that occupied from 1/4 to 1/3 of the total cytoplasmic volume (Figures 90, 91).

Figure 66. Circulating microgametocyte. The parasite is bound by a 3-layered pellicle with a thickened, osmiophilic inner layer (arrow). Other organelles include a nucleus (N), mitochondria (M) with tubular cristae and food vacuoles (Fv) that contain pigment. X 35,500.

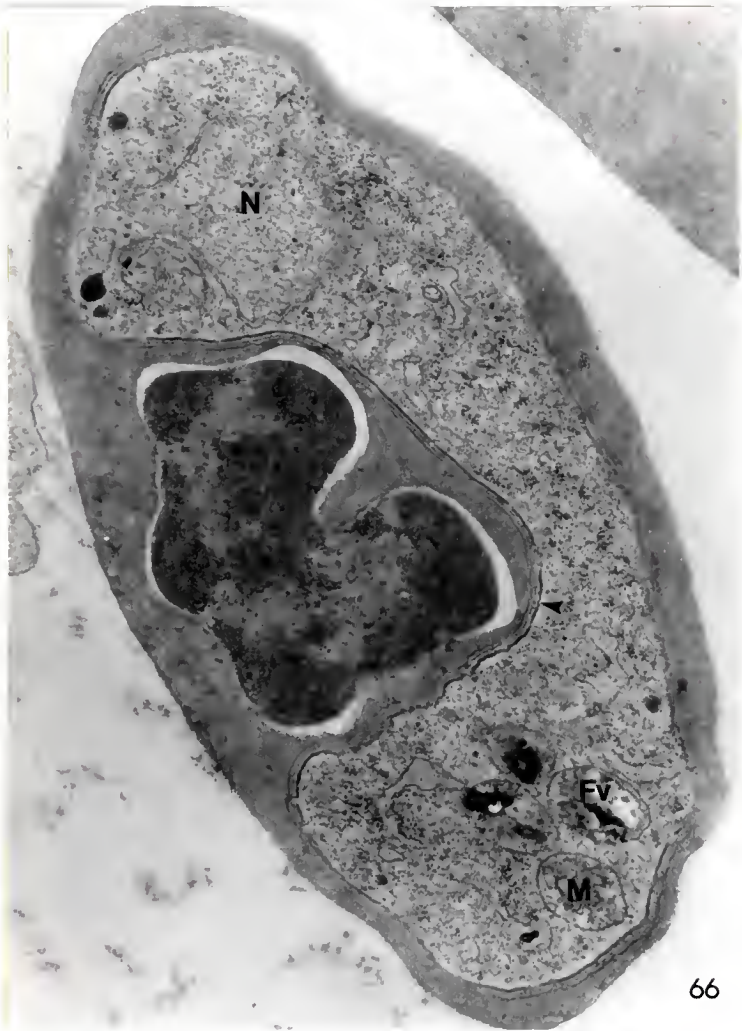


Figure 67. Circulating macrogametocyte. The parasite is bound by a 3-layered pellicle (arrow) and has a branched nucleus (N) with a prominent nucleolus (Nu), mitochondria (M) with tubular cristae and osmiophilic bodies (double arrow). The endoplasmic reticulum (Er) contains a moderately dense, amorphous material. X 46,400.

Figure 68. Higher magnification of Figure 67. The endoplasmic reticulum (Er) is continuous with the inner, osmiophilic layer of the pellicle (arrow). X 72,000.

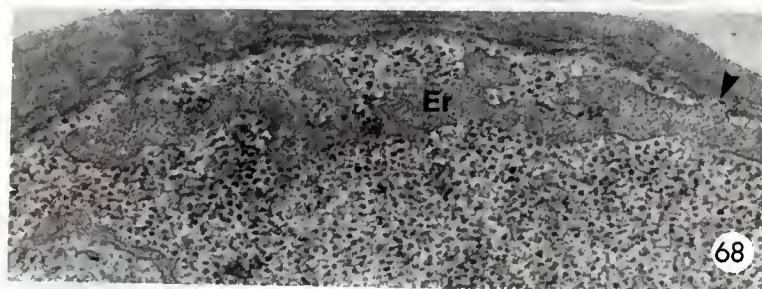


Figure 69. Circulating macrogametocyte. The gametocyte has a cytostome (arrow) with an associated food vacuole (Fv) derived from an indentation of the 2 outer layers of the pellicle (arrow). Older food vacuoles (double arrow) are bound by a single unit membrane and contain diffuse, electron dense masses of pigment (P). X 87,000.

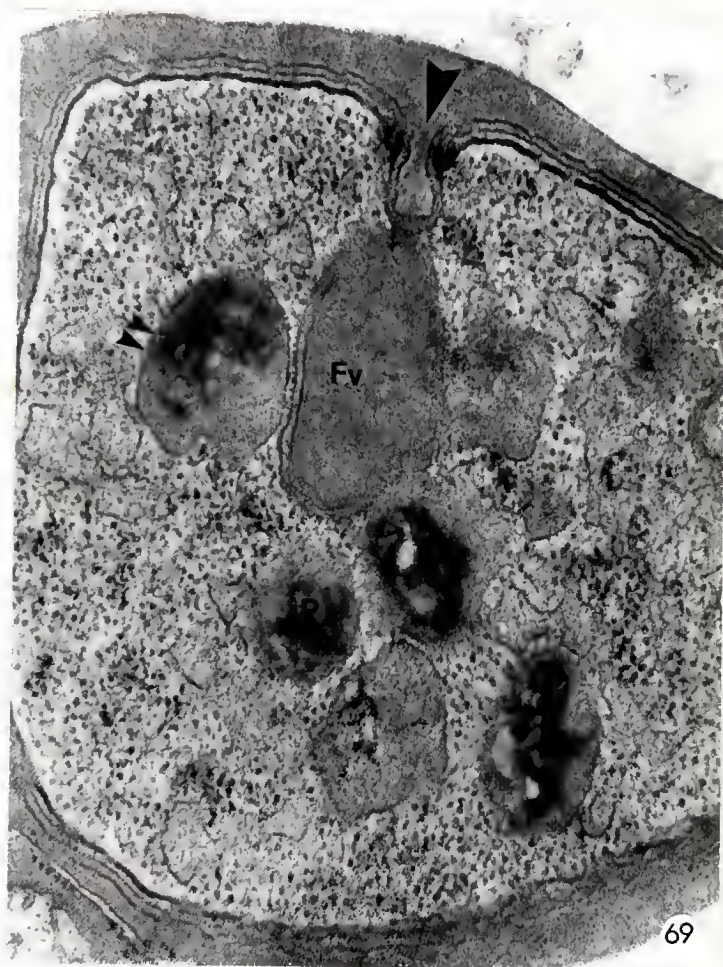


Figure 70. Maturing macrogamete. The outer layer of the pellicle has separated from the parasite and formed membranous coils (arrows). Mitochondria (M) and endoplasmic reticulum filled with amorphous material are scattered in the cytoplasm. X 58,000.

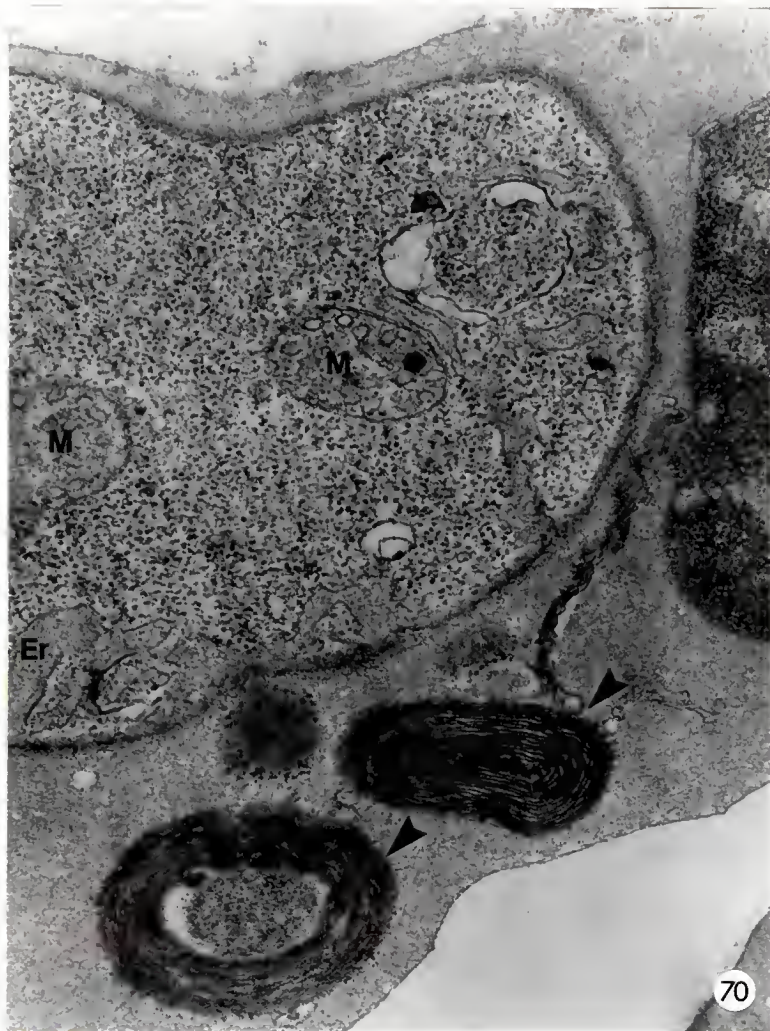


Figure 71. Exflagellating microgametocyte. At the same time the outer layer of the pellicle separates from the parasite (double arrow), breaks appear in the inner osmiophilic layer (arrow). X 46,400.

Figure 72. Higher magnification of Figure 71. X 72,000.



Figure 73. Maturing macrogamete. The outer layer of the pellicle has separated from the gamete and formed membranous whorls that are lined on their exterior by dense granules that are the same size and electron density as free ribosomes (arrow). X 66,700.

Figure 74. Maturing macrogamete. The nucleus (N) is elongate and extends across the diameter of the gametocyte. A nucleolus (Nu) is present at one end of the nucleus and an electron dense mass (large arrow) with faint, embedded microtubules (small arrow) is located at the opposite end. X 58,000.

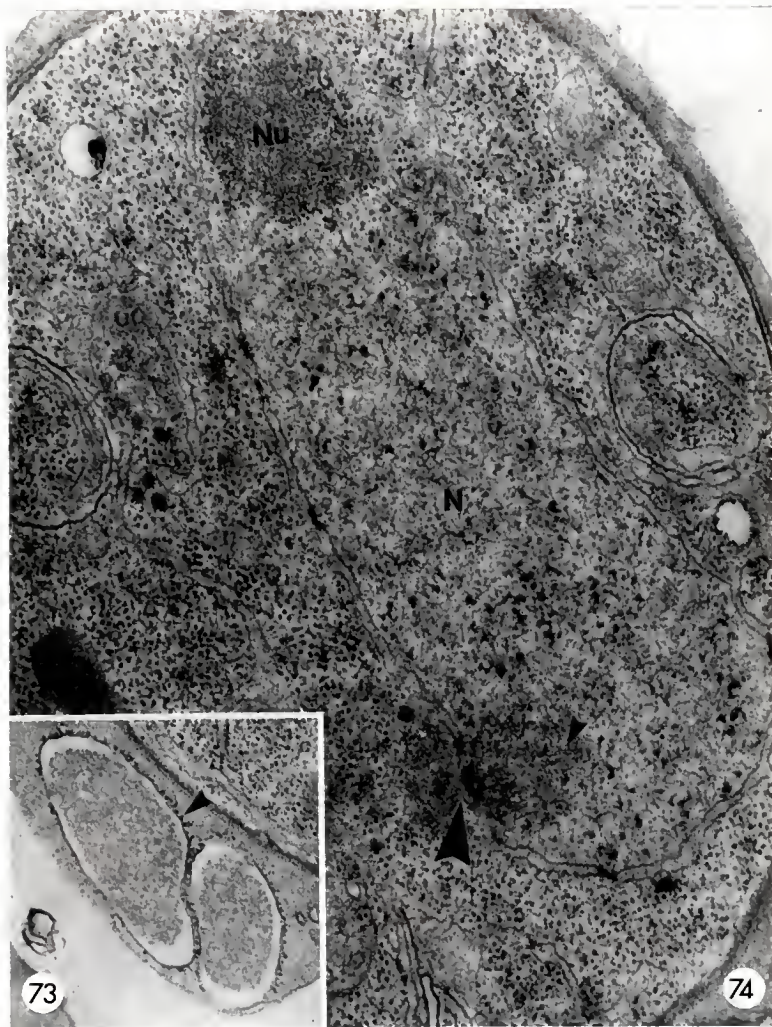


Figure 75. Extracellular maturing macrogamete. The gamete has a pellicle composed of 2 continuous layers (arrow), an elongate nucleus (N) with a nucleolus (Nu), mitochondria (M) and an extensive network of endoplasmic reticulum (small arrows). X 34,800.

Figure 76. A higher magnification of a portion of Figure 75. The macrogamete has a cytostome (large arrow) that is surrounded by 2 electron dense thickenings. The pellicle is composed of 2 uninterrupted unit membranes (small arrows). X 74,750.



Figure 77. Extracellular exflagellating microgametocyte. The remnants of the host, red blood cell nucleus are adjacent to the gametocyte (large arrow). The parasite has a large diffuse nucleus (N) with aggregates of electron dense material with embedded microtubules adjacent to the nuclear envelope (small arrows). Axonemes (A) and mitochondria (M) are scattered in the cytoplasm. X 39,000.

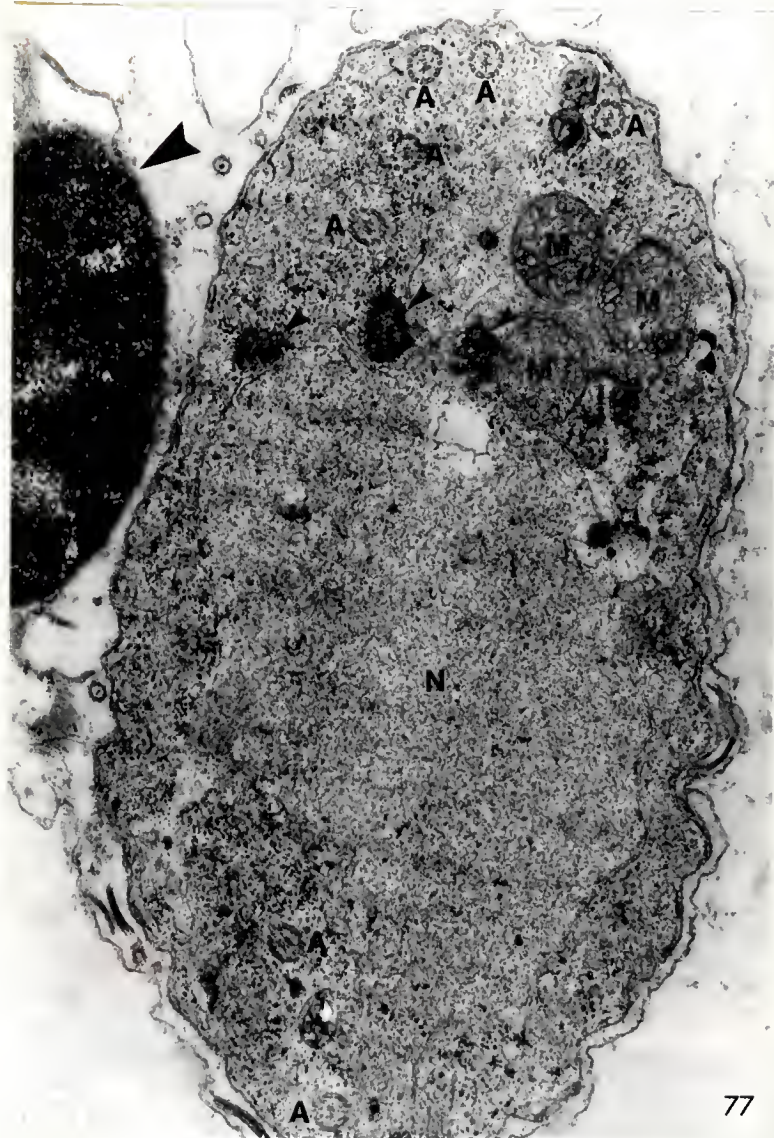


Figure 78. Extracellular exflagellating microgametocyte. Axonemes (A) in various stages of assembly are associated with arm-like extensions (double arrows) of the gametocyte nucleus (N). The gametocyte has a cytostome (large arrow), mitochondria (M) and a 2-layered pellicle with a discontinuous inner layer. X 46,400.

Figure 79. Extracellular exflagellating microgametocyte. The central microtubules (arrows) of the transversely sectioned axoneme have periodic striations along their length. X 66,700.

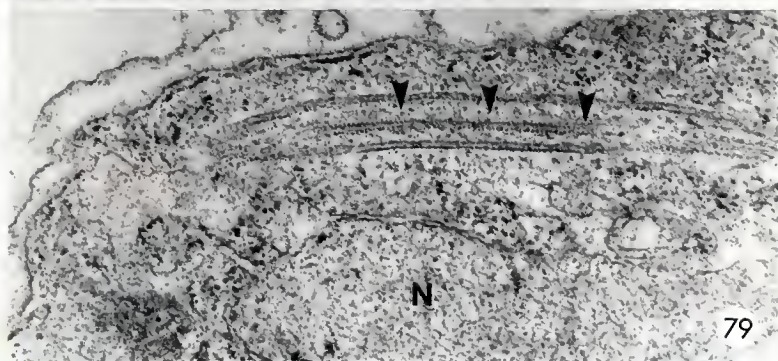
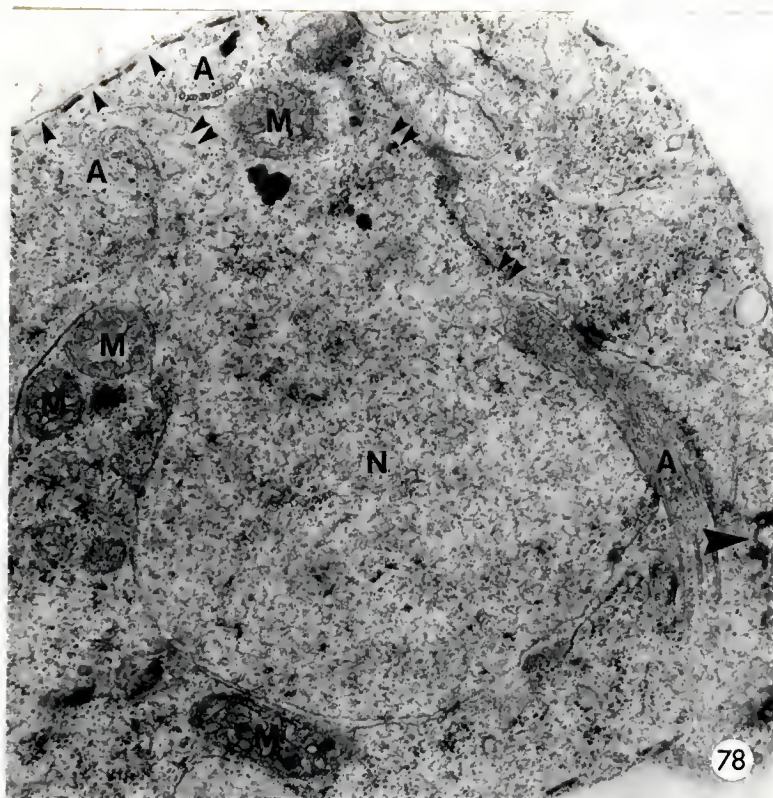


Figure 80. Extracellular exflagellating microgametocyte. Microgametes (large arrows) that contain a single axoneme bud from the outer surface of the microgametocyte, between disruptions (small arrows) in the inner layer of the pellicle. The microgametocyte nucleus (N) is stretched to the base of the budding microgametes. X 58,000.

Figure 81. Extracellular exflagellating microgametocyte. The microgametocyte nucleus is stretched to the base of the budding microgamete (arrow). X 49,200.

Figure 82. Cross sections of microgametes. Each microgamete contains a single axoneme (A) and a mass of nuclear material (N). X 78,000.

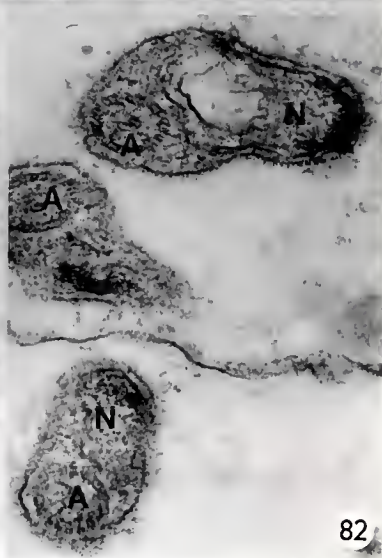
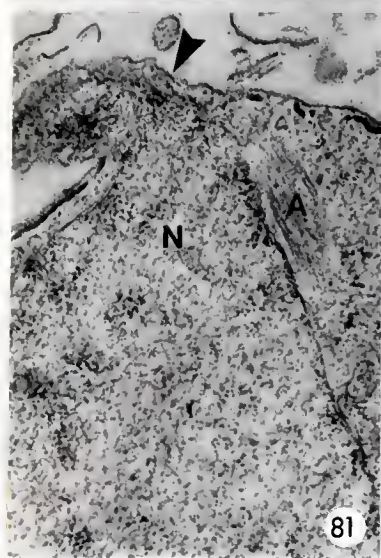
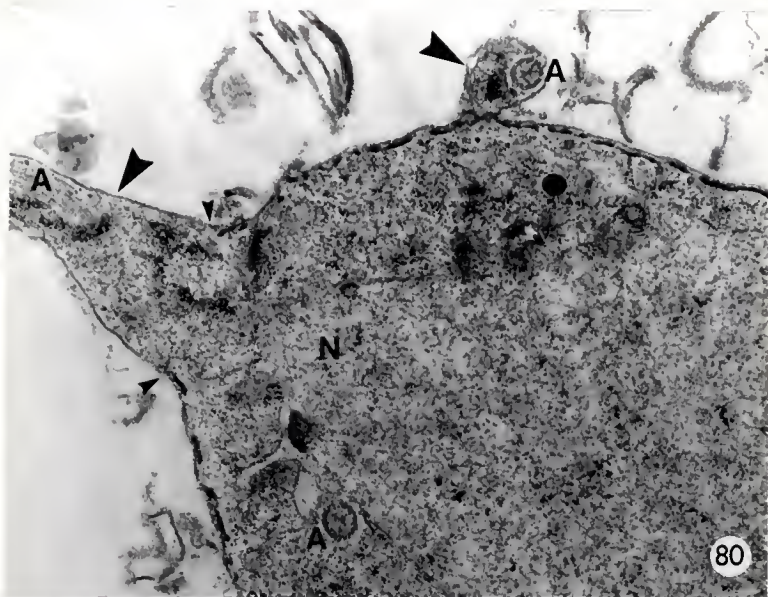


Figure 83. Three-day-old oocyst from a specimen of Culicoides edeni. The oocyst is surrounded by a thick, amorphous wall (large arrows) and is located beneath the basement membrane (Bm) of the midgut. The pellicle of the parasite is underlaid by osmiophilic thickenings (small arrows). Nuclei (N) with prominent nucleoli, large membrane-bound, lipid-like inclusions (L) and mitochondria (M) with tubular cristae are present. X 26,100.



Figure 84. Six-day-old oocyst from a specimen of Culicoides edeni. Budding sporozoites (large arrows) are spaced around the periphery of the sporoblast body. Large nuclei (N) are located at the periphery of the sporoblast body, beneath the budding sporozoites. The remnants of the electron dense apical complex of the ookinete (Ac), as well as large, lipid-like inclusions (L), are near the center of the oocyst. X 26,100.

Figure 85. Six-day-old oocyst from a specimen of Culicoides edeni. Budding sporozoites contain electron dense rhoptries (R), a nucleus (N), a polar ring (small arrow) and subpellicular microtubules (large arrows). The outer layer of the pellicle that surrounds the sporozoites is underlaid by 2 unit membranes in close apposition to one another (double arrow). X 78,300.

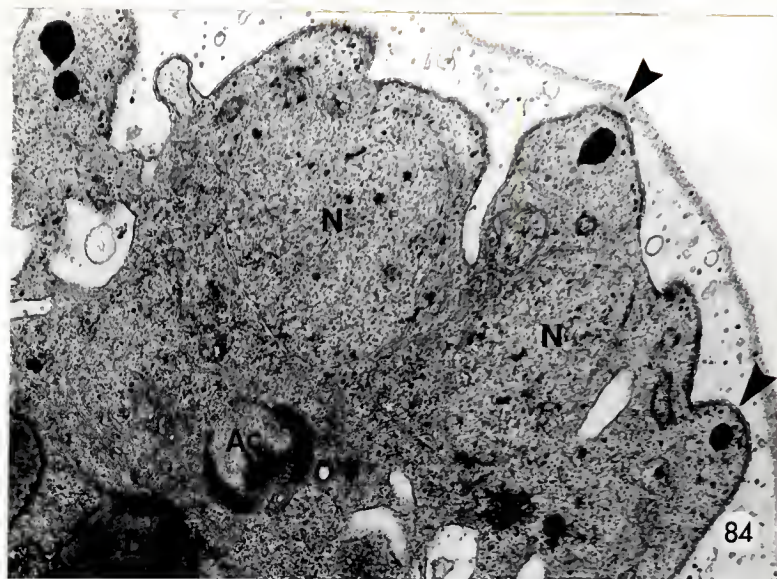


Figure 86. Six-day-old oocyst from a specimen of Culicoides edeni. Budding sporozoites (arrows) contain electron dense rhoptries (R). As they complete their maturation, a small residual body containing large, lipid-like vesicles (L) remains. X 34,800.

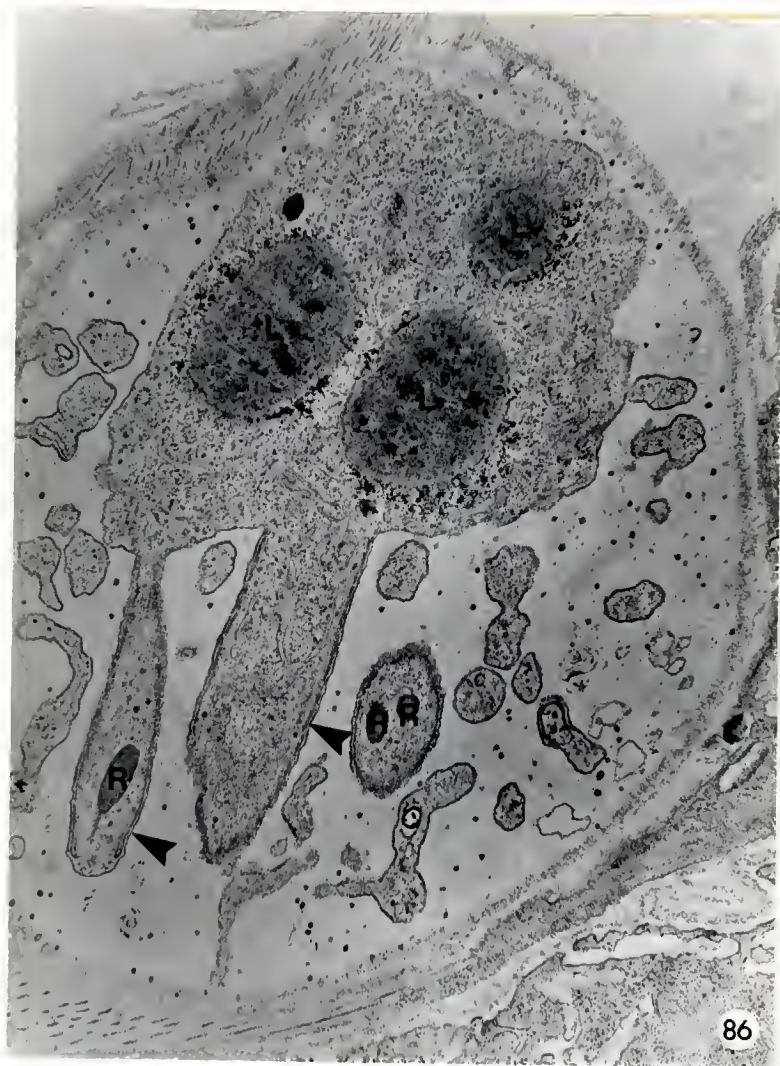


Figure 87. Six-day-old oocyst from a specimen of Culicoides edeni. The oocyst has ruptured, releasing the mature sporozoites. A degenerating residual body (RB) containing lipid-like vesicles (L) and electron dense masses (arrows) remains. X 26,100.

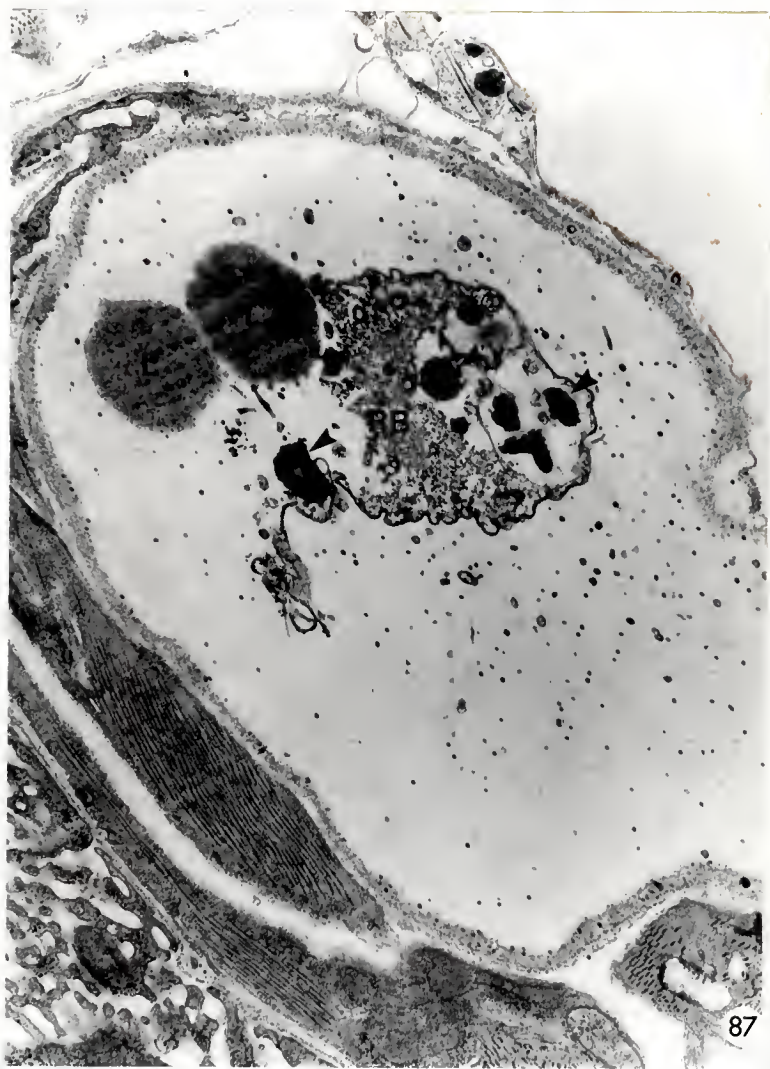


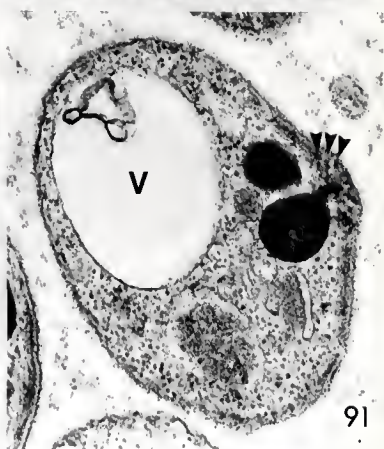
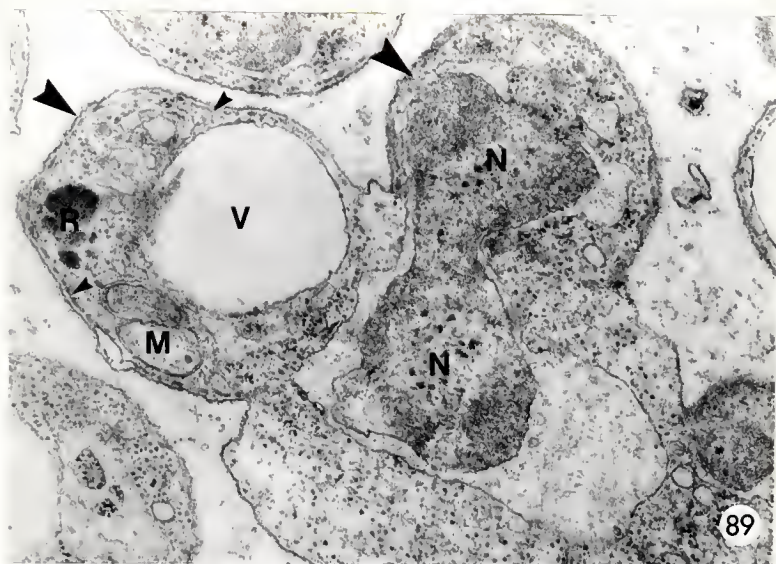
Figure 88. Megaloshizont and associated phagocytic cells (PC) from pectoral muscle of a high dose bird that died spontaneously at 20 days post-infection. The wall (W) of the megaloshizont is thick and laminated. Large quantities of amorphous, granular material are exterior to the wall. A mature merozoite (Me) is located within the interior of the cyst. X 26,100.



Figure 89. A cytomere with budding merozoites (arrows). A nucleus (N) in the process of division is constricted into 2 lobes. Budding merozoites contain a large, electron-lucent vacuole (V), rhoptries (R) and a mitochondrion (M). Merozoites have a pellicle composed of an outer unit membrane and a discontinuous inner layer consisting of 2 unit membranes in close apposition to one another (small arrows). X 58,000.

Figure 90. Mature merozoite. The merozoite has a large, electron-lucent vacuole (V), paired rhoptries (R) and 3 anterior polar rings (arrows). X 58,000.

Figure 91. Mature merozoite. The merozoite has a large, electron-lucent vacuole (V), paired rhoptries (R) and 3 anterior polar rings (arrows). X 58,000.



DISCUSSION

Epizootiology

Vectors

The functional vectors of any arthropod-borne infection must be present at a density sufficient to maintain transmission of the organism, must use the host species as a regular source of blood meals and must be susceptible to development of the parasite (Bates, 1949; Russell, 1959). Of the 29 species of Culiciodes that were captured in New Jersey light traps at Paynes Prairie and Fisheating Creek, individuals of only 12 species took blood meals from turkeys exposed in Bennett traps. Biting collections of 7 of these 12 species, i.e. C. arboricola, C. crepuscularis, C. guttipennis, C. haematopotus, C. hinmani, C. paraensis and C. scanloni, have been made from both birds and mammals (Blanton and Wirth, 1979). Engorged specimens of C. baueri have been collected previously from mammals and specimens of C. ousairani have been collected from birds (Blanton and Wirth, 1979). Biting records for C. edeni, C. knowltoni and C. nanus have not been reported previously.

The total numbers of C. edeni and C. hinmani collected in Bennett traps at Paynes Prairie and Fisheating Creek were considerably larger than collections of any of the other 10 species. While individuals of both species were capable of supporting complete development of the sporogonic stages of H. meleagridis, the greater susceptibility of specimens of C. edeni and their preponderance in Bennett trap collections throughout the year indicate that this species is the primary vector of H. meleagridis in Florida.

Specimens of C. arboricola made up approximately 8% of the collections from Bennett and New Jersey traps at Paynes Prairie. This species may be common enough to play a minor role in the transmission of H. meleagridis in northern Florida. However, total numbers captured at Fisheating Creek were insignificant when compared to the large numbers of C. edeni and C. hinmani that were taken at the same time.

Culicoides knowltoni is found rarely north of central Florida and specimens were not captured at Paynes Prairie. Individuals of this species composed a small fraction (1.4%) of the Bennett Trap catch at Fisheating Creek, but were taken more commonly in New Jersey light traps. This discrepancy suggests that individuals of this species use other hosts as a blood source and may not play a significant role in the epizootiology of H.

meleagridis. Culiciodes crepuscularis is related closely to C. knowltoni and replaces it in northern Florida and throughout North America (Blanton and Wirth, 1979). It is the only species of Culicoides reported from Florida that has been implicated previously as a vector of avian haemoproteids (Bennett and Fallis, 1960). However, specimens of C. crepuscularis were collected rarely in Bennett Traps and light traps at Paynes Prairie and it is unlikely that this species plays an important role in the natural transmission of H. meleagridis in Florida.

Specimens of C. haematopotus were captured too infrequently at Paynes Prairie and Fisheating Creek to implicate this species as a vector of H. meleagridis at either site. However, it is related closely to C. edeni (Blanton and Wirth, 1979). Since the distribution of C. edeni is limited to Florida and the Bahamas (Blanton and Wirth, 1979), C. haematopotus may be an important vector in other parts of North America where the prevalence of H. meleagridis is high.

Sporogonic Stages and Experimental Transmission

The size and morphology of the ookinetes, oocysts and sporozoites of H. meleagridis, as well as the relatively short sporogonic cycle of 6 - 7 days, resemble the findings for other species of Haemoproteus known to develop in ceratopogonids (Fallis and Wood, 1957; Khan and Fallis,

1971; Fallis and Bennett, 1961; Miltgen et al., 1981). The relatively low (50%) infectivity of salivary gland sporozoites from specimens of C. edeni, C. hinmani and C. arboricola as well as the lack of infectivity of sporozoites from the single specimen of C. haematopotus, may be related to the problems inherent in handling sticky salivary glands that are only 100 - 200 um long. Another possibility is that the sporozoites observed in these wild-caught specimens of Culicoides belonged to another species of haemosporidian. Bennett and Coombs (1975) reported a sporozoite prevalence of 13.6% in 184 individuals of C. stilobezziodes from insular Newfoundland. However, only 1 individual (0.5%) out of 210 unengorged, wild-caught specimens of C. edeni from Paynes Prairie was positive for salivary gland sporozoites by dissection, in spite of the fact that collections were made during periods of active transmission of H. meleagridis. Natural infections were not detected in salivary gland dissections of unengorged specimens of C. arboricola or C. hinmani. Attempts to transmit H. meleagridis with individual Culicoides and with pools of several specimens that had been ground in a suitable carrier were more successful.

The 17 day prepatent period of successful experimental infections was less than the 28 day period that Greiner and Forrester (1980) estimated from observations of sentinel

turkeys, exposed in Wild Turkey habitat. It is similar to the shorter prepatent periods of other haemoproteids transmitted by species of Culicoides; 14 - 21 days for H. nettionis, 14 days for H. mansonii and 11-14 days for H. velans (Fallis and Wood, 1957; Fallis and Bennett, 1960; Khan and Fallis, 1971).

Activity Cycles

Most species of Culicoides have crepuscular and/or nocturnal peaks in their daily activity cycles that may play an important role in their contact with potential hosts (Kettle, 1965, 1977; Barnard and Jones, 1980). Kettle (1968b) noted that this cycle is probably an endogenous circadian rhythm, regulated by changes in light intensity and modified by local meteorological conditions such as wind velocity and temperature. A number of sampling techniques have been used to measure diel changes in activity, including truck-mounted interception traps (Bidlemeier, 1961), suction traps (Service, 1971), biting collections (Kettle, 1968a) and paddle traps (Nathan, 1981). Studies that have employed more than 1 sampling method have generally found them to be in close agreement in detecting major peaks of activity during the 24-hour cycle (Nathan, 1981; Service, 1971).

The large, evening Bennett trap catches of specimens of C. edeni, C. hinmani, C. arboricola and C. knowltoni suggest that individuals of these species are primarily crepuscular with major peaks in biting activity near sunset. Since sampling with the Bennett traps was restricted primarily to the 2 hours preceding and following sunset, peaks of activity at other times of the day would have been missed. However, the limited amount of Bennett trapping that was done at night, at dawn and during the day did not detect any major periods of activity.

Peak evening collections of specimens of C. hinmani were consistently and significantly earlier than collections of the other species ($p < 0.05$). Differences among mean capture times for specimens of C. edeni, C. arboricola and C. knowltoni were not consistent from quarter to quarter or site to site. The large amount of overlap among capture times for individuals of these species and the correspondingly large standard deviations for mean capture times at each quarter and site, suggests that the differences are not biologically significant.

The Bennett trap catches and the CO₂-baited suction trap catches of specimens of C. edeni and C. hinmani were similar at Fisheating Creek. Individuals of both species had peaks in their biting/host-seeking activity in the forest canopy during the 2-hour sampling period that

included sunset. Suction trap catches of specimens of C. edeni and C. hinmani fell rapidly in numbers following twilight and remained low until after sunrise, when catches of individual C. hinmani had a second, smaller increase in numbers. Individuals of both species were active at lower levels during the day on 1 or more of the 3 collection dates.

Few specimens of C. edeni and no specimens of C. hinmani were captured in the CO₂-baited suction traps operated near the ground. However, the numbers of individual C. edeni observed on the exposed sentinel turkeys during the day, as well as the high prevalence of transmission of H. meleagridis to sentinel birds caged on the ground, indicates that ground-level biting activity occurred. The ground traps may have been located in a poor position or the technique may not have been sensitive enough to detect low numbers of Culicoides. Snow (1955) noted that biting activity of individuals of C. paraensis, C. spinosus and C. borinqueni spread upward along the main trunk of the tree and then outward into the canopy during the day. Since the ground traps were not positioned near tree trunks, they would not have detected similar movements by specimens of C. edeni or C. hinmani. Other studies have found distinct differences in the vertical distribution of Culicoides species in forest habitat (Snow, 1955; Bennett, 1960; Service,

1971; Tanner and Turner, 1974). In general, ornithophilic species were active in the forest canopy, where avian hosts are presumably more available. Tanner and Turner (1974) suggested that species of Culicoides may occupy a particular vertical stratum and search for a suitable host within that stratum, regardless of whether it is a bird or a mammal.

Wild Turkeys normally roost at night in the middle levels of the forest canopy (Schorger, 1966) and typically fly to a suitable branch at sunset when individuals of C. edeni, C. arboricola and C. knowltoni are becoming active. The significantly higher levels of transmission of H. meleagridis that occurred to sentinel birds exposed in the canopy at Paynes Prairie and Fisheating Creek (Forrester, unpublished data) supports the evidence that individuals of 1 or more of these species serve as vectors of the parasite. Since total numbers of C. hinmani captured in Bennett traps and suction traps were greatest before Wild Turkeys roost for the night and, since this species was never captured in Bennett traps or suction traps operated near the ground, it may play only a minor role in the epizootiology of H. meleagridis.

Transmission and Vector Abundance

Since the discovery of avian haemosporidians by Danilewsky (1889), few epizootiological studies have

been made of any of the avian species of Plasmodium, Leucocytozoon or Haemoproteus. Those that have been conducted have relied primarily on repeated surveys of the host population by blood smears to monitor the prevalence of the parasites under study (Herman, 1938; Herman et al., 1954; Janovy, 1966; Bennett and Fallis, 1960; Klei and DeGiusti, 1975; Greiner, 1975). While this procedure has the advantage of monitoring changes directly in the host population, it is limited by the difficulties and biases inherent in capturing wild birds and diagnosing latent and low intensities of infection. In addition, the precise time when transmission of a blood parasite begins and its relationship to vector populations may be difficult to establish.

Chernin (1952) was one of the first to overcome these problems by using domestic sentinel ducks to monitor natural transmission of Leucocytozoon simondi. Later workers, including Fallis and Wood (1957), Fallis and Bennett (1966) and Herman and Bennett (1976), refined these techniques in more extensive studies of L. simondi and applied them to the study of Haemoproteus nettionis. In conjunction with surveys of wild hosts, the use of sentinel birds provides a powerful tool for studying the dynamics of natural transmission. By decreasing the length of time sentinels are exposed, it is possible to measure the onset of transmission to within days. Sentinel studies are

limited, though, by the availability of a suitable domestic host for the blood parasite under investigation. As a result, this technique has been limited primarily to the study of Leucocytozoon and Haemoproteus in ducks.

Forrester (unpublished data) and Akey (1981), at Fisheating Creek, were the first to use domestic, sentinel turkeys to monitor the natural transmission of H. meleagridis. Earlier surveys in this area by Forrester et al. (1974) established that H. meleagridis had a prevalence that ranged from 70 - 100% in Wild Turkeys older than 5 months. The increasing prevalence of H. meleagridis in juvenile birds collected at monthly intervals up to 1 year after hatching suggested that transmission of the parasite occurred throughout the year (Forrester et al., 1974). In concurrence with this, Forrester (unpublished) found that transmission occurred to 50- 100% of the sentinel poults that he exposed for 12, 2-week intervals between May, 1978 and May 1979. Akey (1981) had similar results during a shorter study between May and August, 1980, in the same area. Both Forrester (unpublished) and Akey (1981) noted that transmission of H. meleagridis occurred at higher prevalences among birds caged in the canopy, than to those caged on the ground. Results of the present study confirm these previous observations at Fisheating Creek and extend the observations from the warm,

subtropical climate of southern Florida to the more temperate climate of northern Florida.

The high prevalence of transmission of H. meleagridis to sentinel turkeys at Fisheating Creek was correlated with the large number of specimens of C. edeni that were captured in light traps and Bennett traps throughout the year. The repeated isolations of H. meleagridis from pools of wild-captured, unengorged specimens of C. edeni provide further evidence of the importance of this species as a major vector of H. meleagridis. The conspicuous absence of individuals of C. hinmani, C. arboricola and C. knowltoni at times when transmission was high, their lower biting rates and the failure to obtain wild isolations of the parasite from individuals of these species, indicates that they may play only minor roles in the epizootiology of H. meleagridis.

The low numbers of specimens of C. edeni that were captured during abnormally cool, wet weather in February and March, 1983 and November, 1984, and the absence of detectable levels of transmission of H. meleagridis may be related. Greiner (pers. comm.) also failed to detect transmission of H. meleagridis to sentinel turkeys in the same area during abnormally wet weather in March and April, 1978. It is unclear whether the population of adult Culicoides was inactive because of cool temperatures

or whether a significant reduction in the population size had occurred.

Fisheating Creek is subject to frequent flooding and wide fluctuations in water levels and flow (U.S. Geological Survey, Water Resources Division, Orlando, Florida). During the drier months of the year (November - March), flow may be reduced to a trickle and the stream can become a series of shallow pools and ponds. During heavy rainstorms, stream levels may rise as much as 7 feet in 24 hours. Because the topography in the area is very flat, ranging from 30 to 55 feet above sea level, the creek swamp may be flooded up to 1 km or more on either side of the stream channel. Since individuals of many species of Culicoides, including C. edeni, breed in moist soil or mud at the edges of permanent or semi-permanent bodies of water (Kettle, 1977), extensive flooding may erode breeding sites and wash the immature stages away. Corresponding reductions in adult populations may reduce the numbers of potential vectors to below the critical density needed to maintain continuous transmission of the parasite.

Patterns of transmission and vector abundance at Paynes Prairie were similar to those at Fisheating Creek. The year-round activity of individuals of C. edeni, the isolation of H. meleagridis from specimens of naturally infected C. edeni, and the conspicuous absence of

individuals of C. hinmani and C. arboricola at times when natural transmission of H. meleagridis was high indicates that C. edeni is the primary vector at Paynes Prairie as well. However, several important differences between the 2 study areas in northern and southern Florida were evident. Transmission of H. meleagridis occurred at a lower prevalence at Paynes Prairie than it did at Fisheating Creek and was not continuous throughout the year (Figures 13, 14, 18). It ceased during the cooler winter months between January and April, when average monthly temperatures were below 60° F, and was often interrupted by periods of 2 or 4 weeks at each of the 2 study sites during the warmer months of the year. In addition, Site A and Site B at Paynes Prairie had significant differences in the time of onset of transmission in the spring, the time when major and minor peaks occurred and the time when transmission ceased in the winter (Figures 13, 14). Unlike the collections at Fisheating Creek, collections of specimens of C. edeni were not closely correlated with peaks in transmission of H. meleagridis at Paynes Prairie (Figures 13, 14).

Censuses of the Wild Turkey population in Florida over the past 30 years have shown dramatic increases throughout the state (Powell, 1967). Surveys have consistently shown that the population in Alachua County, where Paynes Prairie is located, is less than 1/10 as large as it is in

Glades County, at Fisheating Creek (Powell, 1967; L.E. Williams, pers. comm.). In addition, Wild Turkeys from northern Florida have a lower prevalence of H. meleagridis (Forrester et al., 1974). Since many species of Culicoides have flight ranges of less than 1 km (Kettle, 1977), transmission of H. meleagridis at Paynes Prairie may be very local and dependent on the daily movements of Wild Turkeys and their selection of roosting sites for the night. A number of studies have shown that Wild Turkeys maintain home ranges of from 400 - 1500 hectares in the southeastern U.S. and, depending on the season, often move in regular patterns and reuse favorite roosting sites (Schorger, 1966; Bowman et al., 1979). Since Bennett trap collections and light trap collections of C. edeni were of comparable sizes at Sites A and B, the higher prevalences of transmission that were observed at Site B, their earlier peaks and their longer seasonal duration may have been due to greater use of the area by Wild Turkeys. Several authors have reported that Wild Turkeys prefer open woodland, pastures and old fields for foraging and nesting (Stoddard, 1963; Lewis, 1964). Since Site B was located in an ecotone between an open field and the deciduous forest, it may have been more attractive to Wild Turkeys and received greater use. As a result, potential vectors of H. meleagridis would be more likely to become infected with the parasite. The

lower prevalence of sporozoites in naturally infected C. edeni could be a reflection of the smaller Wild Turkey population at Paynes Prairie and the lower prevalence of available gametocytes for potential vectors.

Studies of Plasmodium vivax and P. falciparum have shown that development of the sporogonic stages in mosquitoes is inhibited completely below temperatures of 15 - 20° C (Russell, 1959; Wernsdorfer, 1980). The temperature dependent development of the sporogonic stages is believed to be the major factor limiting the temperate distribution of P. vivax and P. falciparum to areas within the 20° C summer isotherm (Wernsdorfer, 1980). Morii et al. (1965) observed a similar temperature dependence in the development of the sporogonic stages of L. caulleryi. Development of sporozoites in C. circumscriptus and C. arakawae was inhibited at temperatures below 15 - 20° C. They suggested that lower environmental temperatures may be an important factor in the lower prevalences of L. caulleryi in Japanese poultry during the autumn.

Average monthly temperatures at Paynes Prairie and Fisheating Creek are similar between May and October, but range from 3 - 6° C cooler at Paynes Prairie between November and April (Figure 17). The absence of transmission of H. meleagridis during February, March and April, 1983 at Site A, when biting activity of C. edeni

was still high, suggests that the inhibition of sporogony by lower environmental temperatures may be 1 factor limiting the winter transmission of H. meleagridis at Paynes Prairie. During this study, biting activity was never detected for any species of Culicoides below 15o C. Inhibition of host-seeking activity by cool evening temperatures between January and March may also be a major factor in restricting the winter transmission of H. meleagridis.

The epizootiology of H. meleagridis in southern Florida has many similarities to stable, holoendemic malaria (Wernsdorfer, 1980; Gabaldon and Ulloa, 1980). At any given time during the year, most of the Wild Turkey population at Fisheating Creek is infected with H. meleagridis and has circulating gametocytes, available for potential vectors. The high prevalence of H. meleagridis and the large, relatively stable vector (= C. edeni) population insure that a high rate of natural transmission can occur throughout the year. In these respects, the epizootiology of this parasite is significantly different from other avian haemosporidians that have been studied in temperate North America. In temperate locations, populations of vectors as well as the prevalence of avian haemosporidians are highly seasonal. It has been documented that the spring relapse of Haemoproteus, Plasmodium and Leucocytozoon is

under hormonal control and occurs in conjunction with the reproductive cycles of their avian hosts (Haberkorn, 1968; Dessler et al., 1968; Rogge, 1968; Applegate, 1970). Transmission of the parasites occurs during a relatively short period when recently hatched, susceptible young are leaving their nests, when vector populations have increased with the onset of warmer weather and when the older adult population, with chronic, relapsing infections, is available as a reservoir of infection (Janovy, 1966; Fallis and Bennett, 1966).

The more temperate climate at Paynes Prairie, the smaller and more variable vector populations and the lower prevalence of infection in the host population are similar to conditions of hyperendemic, unstable malaria (Wernsdorfer, 1980). Wide variations in the prevalence of infection may occur, depending on environmental conditions, host density and vector abundance and bionomics. However, in contrast to the more unstable, epidemic transmission of other avian blood parasites in northern North America, transmission can remain high throughout most of the year.

PathogenicityExoerythrocytic Development

The description of the schizonts of H. columbae by Aragão (1908) was the first detailed account of the exoerythrocytic development of any haemosporidian parasite (Garnham, 1966). In the 77 years since then, little more has been learned about the exoerythrocytic development of avian haemoproteids. Because experimental infections have been difficult to perform for most species of Haemoproteus, virtually nothing is known about their early stages of exoerythrocytic development.

The results of this study demonstrate that H. meleagridis undergoes at least 2 generations of schizogony in skeletal and cardiac muscle of experimentally infected turkeys. Following intraperitoneal inoculation, sporozoites probably gained access to the circulation via the lymphatic system and became localized in the rich capillary beds of the skeletal muscle. It is unclear whether they began their development within capillary endothelial cells, satellite cells or myofibroblasts. The early schizogonic stages of Sarcocystis may occur in all 3e cell types, as well as macrophages, but are most common in endothelial cells (Entzeroth, 1983; Dubey et al., 1983; Cawthorn et al., 1983; Leek et al., 1977). The early schizogonic stages of other cyst-forming sporozoans,

e.g. Toxoplasma and Hammondia, develop in other organs or tissues (Frenkel and Dubey, 1975; Frenkel, 1973).

The single, 3-day-old schizont that was found appeared to be within the capillary endothelium (Figure 42). However, the presence of disrupted muscle fibers in the infected bird and the development of 5-day-old schizonts both within and between muscle fibers suggests that development may occur in other locations as well (Figures 43, 44, 53).

Studies of other avian haemoproteids have reported development of the mature exoerythrocytic stages in capillary endothelial cells of lung, liver, spleen, heart, kidney and cecum (Khan and Fallis, 1969; Ahmed and Mohammed, 1977; Bradbury and Gallucci, 1971; Sibley and Werner, 1984; Baker, 1966a; O'Roke, 1930; Greiner, 1971). Farmer (1965) found large megaloschizonts within the gizzard muscles of Rock Doves with natural infections of H. sacharovi. He suggested that they may be the exoerythrocytic stages of H. sacharovi, but was unable to transmit the infections with transplants and injections of infected gizzards. Similar forms have not been found in Mourning Doves infected with H. sacharovi (Farmer, 1965; Greiner, 1971) and most workers have ignored the possibility that they may be part of a haemoproteid life cycle. Miltgen et al. (1981) reported the development of megaloschizonts within muscle tissue of

Blossum-Headed Parakeets, naturally infected with H. desseri. They noted that development appeared to be within muscle fibers, rather than cells of the reticuloendothelial system. In their study, as well as the one by Farmer (1965), the megaloschizonts were too large to precisely determine the type of host cell.

Mature, 5-day-old, first-generation schizonts of H. meleagridis contained elongate zoites that differed in size and morphology from the pre-erythrocytic merozoites of mature, 17-day-old, second-generation megaloschizonts (Figures 44, 50). They were approximately the same size as first-generation hepatic schizonts of L. simondi, but lacked the hypertrophied host cell nucleus that is characteristic of Leucocytozoon infections (Huff, 1942). In addition, first-generation merozoites of Leucocytozoon are oval to round in shape, rather than elongate (Desser, 1967, 1974; Akiba et al., 1971; Huff, 1942). The first-generation schizonts of H. meleagridis were more similar in morphology to the first-generation schizonts of Sarcocystis (Leek et al., 1977; Dubey et al., 1983). The small size of schizonts found in the 8-day-old infection suggests that the first-generation schizonts ruptured between 5 and 8 days post-infection and released zoites that initiated a second-generation of schizogony.

Failure to detect developing schizonts at 11 days post-infection may have been because the infected bird had an infection of low intensity. The large difference in size between the 8-day-old and the 14-day-old forms makes it unlikely that a third generation of schizogony could have taken place in other tissues.

The large size of the second-generation megaloschizonts of H. meleagridis is comparable to megaloschizonts that have been found in birds with natural infections of H. garnhami, H. desseri and H. sacharovi (Garnham, 1966; Miltgen et al., 1981; Farmer, 1965). However, the megaloschizonts of H. garnhami contained cytomeres, separated from each other by distinct septa, lacked a thick, hyaline wall and have not been found in muscle tissue (Garnham, 1966). Miltgen et al. (1981) described "pseudo-septa" in immature megaloschizonts of H. desseri. The authors suggested, though, that these were artifacts caused by contraction of the surrounding muscle fibers during fixation of the tissue. The presence of a hyaline cyst wall around megaloschizonts of H. desseri, the absence of septa in mature forms and the exclusive development in muscle tissue were very similar to H. meleagridis. The thick-walled, aseptate cysts described by Farmer (1965) were also very similar to mature megaloschizonts of H. meleagridis, but their development appeared to be limited to gizzard muscle. Immature

megaloschizonts were not found (Farmer, 1965). Since sequential observations of the development of H. garnhami, H. desseri and the forms described by Farmer (1965) have not been made, the significance of cytomere development in megaloschizonts of H. meleagridis cannot be determined. The progressive formation of smaller and smaller cytomeres is most similar to that described for megaloschizonts of Leucocytozoon (Huff, 1942; Khan and Fallis, 1970). However, final merozoite formation in species of Leucocytozoon occurs by fragmentation of the cytomere cytoplasm rather than by bud formation (Desser, 1970).

Cytomere formation has not been confirmed in other species of Haemoproteus. Aragão (1908) described the segmentation of multinucleated schizonts of H. columbae in lung tissue into numerous uninucleate masses that underwent further nuclear division and growth. Wenyon (1926) termed these masses cytomeres. Other studies have not observed this process during the exoerythrocytic development of H. columbae (Ahmed and Mohammed, 1977; Mohammed, 1965; Bradbury and Gallucci, 1971). Ultrastructural studies by Bradbury and Gallucci (1972) revealed that multinucleated masses of cytoplasm developed as clefts and projections from the parent schizont. Since these did not develop from discrete, uninucleate masses and did not always detach from the parent body, Bradbury and Gallucci (1972) used the term "pseudo-cytomere" as used by

Garnham (1951) to describe them. Bray (1960) attempted to resolve differences between the terms "cytomere" and "pseudo-cytomere" and simplify terminology by redefining cytomeres as "separate growing nucleated masses, produced within a process of schizogony by the division of a schizont.". He stated that the process "may produce further cytomeres but produces uninucleated organisms as an end point." Unfortunately, the redefinition has not been widely accepted. Most authors have applied the term "pseudo-cytomere" to descriptions of schizonts that contain multinucleated masses of protoplasm that remain attached to the parent schizont (Bradbury and Gallucci, 1972; Sterling and DeGiusti, 1972). Since knowledge of the entire developmental sequence is required to determine whether a multinucleated mass developed from a separate uninucleate body or from a multinucleated mass that detached late in development from the parent body, use of either term may be misleading when the complete morphogenesis is unknown.

During the development of second-generation megaloschizonts of H. meleagridis, cytomere formation occurred between 8 and 14 days post-infection. At the present time it is not possible to determine whether the cytomeres developed by segmentation of the multinucleate 8-day-old schizonts into uninucleate masses or by detachment of multinucleated masses from the parent body.

Since 14-day-old megaloschizonts contained numerous discrete bodies that resembled the cytomeres described in developing megaloschizonts of Leucocytozoon, the terminology as defined by Bray (1960) has been applied to megaloschizonts of H. meleagridis.

The severe lameness and extensive myopathy that occurred in infected birds during development of the small, first-generation schizonts of H. meleagridis is unusual for haemosporidian infections. The early, pre-erythrocytic stages of most species of Plasmodium do not elicit any host reaction and later exoerythrocytic schizonts appear to be pathogenic only during unusual circumstances when the parasite is exceptionally infectious to the host (Huff, 1969). Little host reaction has been observed in association with the small endothelial schizonts of H. fringillae, H. columbae, H. palumbis, H. lophortyx, H. nettionis and H. maccallumi (Khan and Fallis, 1969; Mohammed, 1965; Ahmed and Mohammed, 1977; Baker, 1966a; O'Roke, 1930; Sibley and Werner, 1984; Greiner, 1971). Garnham (1966) reported cellular infiltration similar to acute interstitial pneumonia in the alveolar septa of Rock Doves with heavy, early infections of H. columbae, but did not provide any details of the infection. Of the 3 genera of avian haemosporidians, the exoerythrocytic stages of Leucocytozoon have been documented as the most pathogenic (Lund and Farr, 1965). However, most pathology

is associated with development of the large megaloschizonts (Miller et al., 1983; Newberne, 1957).

During the earliest stages of infection with H. meleagridis, myopathy was restricted to isolated muscle fibers. By 5 days post-infection, entire bundles of as many as 5-10 adjacent myofibers were necrotic. The absence of focal areas of inflammation and necrosis immediately around first-generation schizonts suggests that the extensive necrosis was not due to the release of toxins by developing parasites or to blockage or interference with the local circulation. The extensive myopathy may have been related to the size of the initial inoculum. The total numbers of exoerythrocytic schizonts were significantly fewer than in the pathogenicity experiment where the total sporozoite dose was only 1/3 as large. Perhaps many host cells were invaded by more than 1 sporozoite and were unable to support the development of multiple schizonts. Their early death would explain the low number of megaloschizonts that developed relative to the infective dose. Wallace (1973) observed myositis and myocarditis in mice that had been infected orally with large numbers of Sarcocystis sporocysts. He found that the myositis became apparent before sarcocysts developed and was not associated with the early first-generation schizonts. The myositis was also evident in experimentally infected mice that failed to develop detectable sarcocysts,

suggesting that the early inflammatory reaction may have been to dead and dying host cells and parasites.

The host response surrounding 14-day-old and 17-day-old second-generation megaloschizonts is similar to the host reactions to the large intramuscular cysts of Sarcocystis. Leek et al. (1977) noted multifocal perivascular inflammatory infiltrates in the musculature of lambs that were experimentally infected with Sarcocystis. The response was not associated with developing sarcocysts, but was apparent around degenerating cysts. Mundy et al. (1975) reported similar results. Other studies of Sarcocystis and Hammondia have reported the necrosis and mineralization of muscle fibers adjacent to developing cysts (Cawthorn et al., 1984; Frenkel and Dubey, 1975).

Among haemosporidian parasites, the host response to megaloschizonts of H. meleagridis is similar to host reactions to L. simondi. Miller et al. (1983), Desser (1967), Cowan (1957) and Newberne (1957) reported the presence of mixed inflammatory infiltrates composed of mononuclear cells, heterophils, plasma cells and red blood cells around both intact and ruptured megaloschizonts as well as necrotic changes in the surrounding host tissue. Cowan (1957) and Miller et al. (1983) described the spontaneous necrosis of Leucocytozoon megaloschizonts in the absence of invading host cells. In both studies, necrotic megaloschizonts were filled with an

amorphous, eosinophilic material that resembled the material observed in necrotic megaloschizonts of H. meleagridis.

The megaloschizonts described by Nair and Forrester (unpublished) in skeletal muscle of a naturally infected Wild Turkey are identical in structure and size to megaloschizonts of H. meleagridis from experimentally infected turkeys. Many of the cysts observed by Nair and Forrester contained disorganized masses of basophilic material. Since the tissue had been frozen for several weeks prior to fixation, degenerative changes in the megaloschizont morphology may be an artifact of the manner in which the tissue had been handled. The discovery of these forms in a naturally infected Wild Turkey indicates that the site of development and the associated host response are not artifacts related to the way the experimental turkeys were infected.

Miltgen et al. (1981) noted the similarity between the exoerythrocytic stages of H. desseri and Arthrocystis galli, an organism of uncertain taxonomic status described by Levine et al. (1970) in chickens from India, and suggested that the 2 organisms may be synonymous. Other descriptions of cyst-forming organisms have been reported from a variety of avian hosts in several families of birds. Most infections have been characterized by the presence of large, intramuscular schizonts, similar in

morphology to the megaloschizonts of Leucocytozoon (Gardiner et al., 1984). In most cases, development of megaloschizonts occurred in the absence of circulating gametocytes. Garnham (1973a, 1973b) suggested that these were aberrant Leucocytozoon infections in abnormal hosts. Gardiner et al. (1984) recently reviewed these reports and described similar megaloschizonts in pen-reared Northern Bobwhites from California. The cysts described in all the reports have a number of features in common, including a hyaline wall of variable thickness, development in muscle tissue and formation of numerous, spherical merozoites approximately 1 μ m in diameter. Most have an associated host myopathy.

The results of this study provide the first experimental evidence that some of these organisms may be haemoproteids. Gardiner et al. (1984) observed pigmented gametocytes, morphologically similar to H. lophortyx, in red blood cells of Northern Bobwhites that died from an extensive myositis associated with developing megaloschizonts. However, since they observed small schizonts with elongate zoites and hypertrophied host cell nuclei in spleen tissue that were unlike any reported schizonts of Leucocytozoon or Haemoproteus, they suggested that the causative organism may be a new member of the Haemosporina. O'Roke (1930) conducted a detailed study of H. lophortyx in California Valley Quail, but never reported

similar lesions. Instead, he found small schizonts in endothelial cells of lung tissue. It is not clear from his work, though, whether he examined histological sections of skeletal, cardiac and gizzard muscle. The absence of obvious gross lesions and cysts in the turkey with a 17-day-old infection of H. meleagridis suggests that megaloschizonts may be missed, particularly if their development is not suspected. Since muscle tissue makes up a large proportion of the total body mass, small numbers of scattered cysts may be difficult to find and yet produce enough gametocytes to infect a large proportion of the red blood cells.

The elongate, first-generation merozoites of H. meleagridis are similar to the merozoites contained in small schizonts described by Gardiner et al. (1984). The development of schizonts in the spleen of 1 turkey during the pathogenicity experiment indicates that the exoerythrocytic stages found by Gardiner et al. (1984) may be part of the life cycle of H. lophortyx. Since haemoproteids are believed to be specific to host family (Bennett et al., 1982), Northern Bobwhites may be a susceptible but aberrant host for H. lophortyx. Clearly, additional experimental work with other species of Haemoproteus is needed to determine the significance of these exoerythrocytic stages in haemoproteid life cycles.

Pathology

Experimental infections of Haemoproteus meleagridis produced a moderate to severe myositis and myocarditis in domestic turkeys. The inflammatory reactions were associated with the development of first and second-generation schizonts. The lameness exhibited by infected birds and the gross and microscopic lesions were similar to naturally acquired infections of Arthrocystis galli (Levine et al., 1970; Opitz et al., 1982). Both Levine et al. (1970) and Opitz et al. (1982) noted extensive muscle necrosis, inflammation and hemorrhage around the megaloschizonts. Opitz et al. (1982) also observed dystrophic calcification of necrotic muscle fibers and the formation of scar tissue. Neither Opitz et al. (1982) or Levine et al. (1970) reported the thrombus formation or circulatory disturbances that were observed in this study. It is likely that they developed in association with the acute and chronic inflammatory responses to intact and ruptured megaloschizonts and the associated necrotic changes in surrounding muscle fibers.

The distribution and morphology of second-generation megaloschizonts observed in the pathogenicity experiment was identical to megaloschizonts found during earlier experimental infections. In addition, small, thin-walled schizonts that contained both elongate

zoites and small, spherical pre-erythrocytic merozoites were found in the spleen of 1 high dose bird that died spontaneously. Other studies have reported the development of exoerythrocytic schizonts of Plasmodium and Leucocytozoon in reticular cells of the spleen (Huff, 1969; Akiba et al., 1971). Host cell nuclei of the reticular schizonts were not hypertrophied as is characteristic of Leucocytozoon infections.

The reductions in growth and weight gain in experimentally infected birds were dose dependent and most pronounced between 1 and 3 weeks post-infection, during development of second-generation megaloschizonts (Figures 37, 38). The onset of lameness and anorexia in the high dose birds was approximately 1 week later than in the earlier series of experimental infections and was probably associated with the inflammatory response to the second-generation megaloschizonts. Because high dose birds received fewer than 1/3 as many sporozoites as birds that were infected to study exoerythrocytic development, pathological changes associated with development of the first-generation schizonts may not have been as severe.

Following the crisis, all infected birds improved. This was most evident among turkeys in the high dose group that exhibited little significant weight gain between weeks 0 and 2, between weeks 1 and 3 and between weeks 3 and 4

post-infection (Figure 37). The surviving high dose birds remained significantly smaller than control and low dose turkeys throughout the course of the study (Figure 38). Low dose birds were smaller than controls, but not significantly so. If the sample size had been larger, it is possible that significant effects on growth and weight gain would have been detected for the low dose group as well.

Few host effects were associated with the development of the erythrocytic gametocytes, although these may have been masked by the massive host response to the megaloschizonts. Average hematocrit and hemoglobin values were not significantly different for any of the 3 experimental groups at the crisis, or at the second peak in parasitemia at 6 weeks post-infection (Figures 39, 41). A significant drop in average hemoglobin concentration occurred in the high dose group, 1 week after the crisis. This drop corresponded to the rapid clearance of parasitized red blood cells from the circulation and their replacement with immature erythroblasts that had not completely synthesized their total hemoglobin content (Lucas and Janroz, 1961). The absence of other significant weekly differences in average hemoglobin concentration and average hematocrit among the 3 experimental groups indicates that removal of parasitized red cells was balanced by the synthesis and release of erythroblasts.

When comparisons of average hematocrits were made by group, the same trends were evident in each group, indicating that significant differences within groups may be related to the age of the birds (Figure 39).

Deposition of pigment in macrophages of the spleen, liver and lungs began at approximately 22 DPI, when maturing erythrocytic gametocytes began to develop detectable pigment granules. The clearance of parasitized erythrocytes from the circulation was probably accomplished by phagocytic activity of these cells, as has been described in infections of Plasmodium (Taliaferro, 1941). Most pigment was deposited in the spleen, where the major elimination of the parasite population from the peripheral circulation occurs (Taliaferro, 1941). Follicular hyperplasia and enlargement of the spleen, characteristic of other species of Haemoproteus and Plasmodium, also occurred (Becker et al, 1956; Russell et al, 1943; Taliaferro, 1941).

The significant drop in plasma protein levels in the low dose and high dose birds at 1 week post-infection may have been related to the increase in vascular permeability that accompanies acute inflammatory responses (Figure 40) (Smith et al., 1972). Mahrt and Fayer (1975) did not find significant changes in total serum protein during experimental infections of calves with Sarcocystis fusiformis. However,

Leek et al. (1977) observed a significant drop in total serum protein in lambs infected with Sarcocystis. The decrease occurred during development of first-generation schizonts in endothelial cells. Leek et al. suggested that the drop in serum protein levels resulted from glomerulonephritis associated with development of schizonts in endothelial cells of the kidneys. Similar lesions were not detected in infected birds examined in this experiment.

The increase in plasma protein concentrations in the high dose group at 2 weeks post-infection may have been related to dehydration observed among these turkeys (Figure 40). Augustine (1982) noted similar increases in plasma protein concentrations when turkeys were deprived of water for periods of up to 72 hours. When birds were deprived of both food and water, significant changes in plasma protein concentrations did not occur. Since feed consumption was not measured in the pathogenicity experiment, it is unclear whether the high dose birds ceased eating and/or drinking completely. The diarrhea associated with the concurrent Salmonella infection may have acted in conjunction with decreases in food and water consumption to dehydrate the birds and concentrate total plasma proteins.

The increase in average plasma protein concentration at 5 and 6 weeks post-infection may reflect the synthesis of immunoglobulins (Figure 40). Other studies of avian species of Plasmodium and

Leucocytozoon have documented increases in parasite specific immunoglobulins as the infections progressed (Congdon et al., 1969; Morii, 1972).

By 8 weeks post-infection, when the experiment was ended, surviving birds had regenerating muscle fibers and scarring associated with the destruction and removal of megaloschizonts (Figures 33, 34, 35). The persistence of some megaloschizonts up to 8 weeks post-infection suggests that they may be a source for relapses.

It is impossible to determine whether the high (33%) mortality in the high dose group resulted from the H. meleagridis infection alone, or whether the concurrent Salmonella infection was a significant factor. Studies of the interactions between P. berghei and Salmonella typhimurium have shown that mice infected with both agents died earlier than mice infected with either agent alone (Kaye et al., 1965). Viens et al. (1974) had similar results when they infected mice with P. yoelii and Bordetella pertussi. Cox (1978) suggested that the synergism between Plasmodium infections and other infectious agents may result from the immunodepression that often accompanies Plasmodium infections. Similar studies have not been conducted with Haemoproteus.

Because of the way they were housed, all birds in each of the 3 experimental groups had an equal exposure to Salmonella enteritidis. The close proximity of the battery

cage compartments to one another, the inevitable fecal contamination that occurred in the food and water and successful isolation of Salmonella from representatives of each experimental group suggests that all the birds were infected. Since birds vary in their output of Salmonella organisms from day to day, the low number of Salmonella isolations from cloacal swabs of each group at 4 and at 8 weeks post-infection is not surprising (Williams, 1978). It is significant that the only birds to develop clinical salmonellosis were those in the high dose group. They exhibited signs of infection between 12 and 28 days post-infection when stress from the H. meleagridis infection reached its peak. Perhaps the Haemoproteus infection weakened the high dose group sufficiently to make them susceptible to Salmonella and other secondary bacterial and fungal infections as well. Salmonella is frequently isolated from commercial feeds that use animal products to boost protein levels (Williams, 1978). It is likely that the turkeys used in the experiment acquired their infections from the unmedicated game bird chow they were fed.

Most authors have considered Haemoproteus to be a relatively benign parasite (Bennett et al., 1982; Kemp, 1978; Fallis and Desser, 1977; Levine, 1961). Considering how prevalent Haemoproteus is in many bird populations (Bennett, 1982), the few isolated reports of pathogenic

effects in natural Haemoproteus infections have done little to refute this view. The best documented pathology in natural Haemoproteus infections was done by O'Roke (1930) with his study of H. lophortyx in California Valley Quail. He attributed the morbidity and mortality he observed to anemia caused by rupture of parasitized erythrocytes and felt that the gametocytes made their host cells more fragile and susceptible to rupture as they passed through the capillary beds. The results of this study indicate that H. meleagridis can be severely pathogenic in high doses and can have detectable effects on growth and weight at low doses. The pathogenic effects associated with the experimental infections was more similar to host reactions to megaloschizonts of Leucocytozoon than to the erythrocyte destruction and anemia caused by Plasmodium.

Since the lesions in the naturally infected Wild Turkey found by Nair and Forrester were similar to those of the experimentally infected birds, H. meleagridis may be a cause of morbidity and mortality in Wild Turkeys. It may only be significant in holoendemic areas such as southern Florida and perhaps southern Texas where the prevalence of the parasite ranges from 90-100% and rates of transmission are high (Forrester et al., 1974; Cook et al., 1966).

The low dose birds were infected with the sporozoites contained in 5 infected C. edeni. A number of studies of Plasmodium have shown that mosquitoes may inoculate only a small percentage of their total number of salivary gland sporozoites when they take a blood meal (Vanderberg, 1977). Similar studies have not been done with ceratopogonids. However, since the prevalence of H. meleagridis approaches 2% of the population of nulliparous C. edeni at Fisheating Creek and since a bird may be bitten by several thousand specimens of Culicoides in a single night, it is possible that intensities of natural infection comparable to the low dose may be reached over a period of several weeks. Additional research on the effects of repeated, low level exposure of H. meleagridis to pen-reared Wild Turkeys would be helpful in determining the possible significance of this parasite in the Wild Turkey population.

Host Specificity

The present taxonomy of avian species of Haemoproteus is based on the limited experimental evidence that suggests that some species are specific to host family (Bennett et al., 1982; Bennett et al., 1972). While this taxonomic scheme provides a functional framework for dealing with the

large number of often poorly described species in this genus, it is totally dependent on the maintenance of a stable system of classification of the avian hosts. Recently, the Committee on Classification and Nomenclature of the American Ornithologist's Union (1983) published a revised sixth edition of the Checklist of North American Birds. For the first time since the publication of the third edition in 1910, the committee incorporated major changes in the systematics of higher categories. These changes were based on the conservative evaluation of many new discoveries in the morphology, paleontology, biochemistry, genetics and behavior of avian species (Checklist of North American Birds, 1983). While the status of many orders and families remained unchanged, extensive revisions were made in others, e.g. Passeriformes, Galliformes, that may require corresponding revisions in haemoproteid taxonomy.

Prior to the 1983 revision of the Checklist of North American Birds, the Order Galliformes was composed of 5 families: Cracidae (Curassows and Guans), Phasianidae (Pheasants and Quail), Tetraonidae (Grouse), Meleagrididae (Turkeys) and Numididae (Guineafowl). In the 1983 edition of the Checklist of North American Birds, only 2 families were recognized in the Order Galliformes: Cracidae (Curassows and Guans) and Phasianidae (Pheasants, Quail, Grouse, Turkeys, Guineafowl). The families Phasianidae,

Tetraonidae, Meleagrididae and Numididae were lowered to subfamily status and the quail were separated from the pheasants and elevated to form the subfamily Odontophorinae.

Prior to the major taxonomic revisions in the Order Galliformes, results of the host specificity experiment would have invalidated assumptions about the host specificity of avian haemoproteids and provided evidence that the current taxonomy of the genus Haemoproteus may be flawed. Instead, the finding that H. meleagridis can be transmitted experimentally between Turkeys (subfamily Meleagridinae), Chuckars (subfamily Phasianinae, tribe Perdiciini) and Ring-necked Pheasants (subfamily Phasianinae, tribe Phasianini), does not invalidate the basic assumptions of current haemoproteid taxonomy. Unfortunately, a true test of the taxonomic scheme was not conducted since avian hosts outside of the current Phasianid family were not included in the experiment. However, the conflicting interpretations, dependent on host classification, illustrate the dangers inherent in basing the taxonomy of 1 unrelated group of organisms on that of another.

The results of the host specificity experiment provide some support for the current revisions within the Order Galliformes. Nolan et al. (1975) found that rabbit antibodies to 9 proteins purified from domestic

chickens reacted as well to their protein counterparts from turkeys as they did to their protein counterparts from Ring-Necked Pheasants. They briefly reviewed other evidence from hybridization experiments, chromosome studies, electrophoretic and immunological experiments and anatomical studies that supported the similarities between turkeys and other Phasianids. Since cell penetration by some sporozoan parasites may be mediated by highly specific cell surface receptors (Miller et al., 1978), the parasites can, in a sense, be considered as highly specific probes. The successful experimental transmission of H. meleagridis from a turkey to a Ring-necked Pheasant and a Chukkar suggests that all 3 species may have similar cell surface receptors. While these similarities may be of minor taxonomic significance, they support the data showing close biochemical similarities among these species.

The family Phasianidae presently contains members of 4 former avian families. Prior to the revision, Bennett et al. (1982) reported 8 valid species of Haemoproteus from the family Phasianidae - H. chuckari, H. chapini, H. ammoperdis, H. santosdiasii, H. bambusicolae, H. lophortyx, H. pratasi and H. rileyi, 2 valid species of Haemoproteus from the family Numididae - H. pratasi and H. silvai, 2 valid species from the family Tetraonidae - H. mansonii (= H. canachites) and H. stableri and 1 valid species from the family Meleagrididae - H. meleagridis.

Four species, H. chukari, H. coturnix, H. gallinarum and H. perdix, were reported as nomen nuda. Bennett et al. (1982) lists H. balfouri from Guinea fowl as valid, but descriptions and figures of this species are absent from the cited references. It will also be considered a nomen nudum. Under the current system of haemoproteid taxonomy, these species must be morphologically distinct from one another to retain their status as separate taxa.

Haemoproteus meleagridis is most similar to H. mansonii and H. stableri of Grouse (subfamily Tetraoninae). Gametocytes of other species of Haemoproteus from the subfamilies Phasianinae and Numidinae are halteridial and only partially encircle the host cell nucleus. The geographical range of H. mansonii overlaps most of the geographical range of H. meleagridis, while H. stableri has been reported only from Ruffed Grouse in Montana (White and Bennett, 1979). Greiner and Forrester (1980) discussed the similarities between H. mansonii and H. meleagridis and stated that the number of pigment granules in circumnuclear gametocytes of H. mansonii was significantly fewer than in circumnuclear gametocytes of H. meleagridis. They also noted that the number of pigment granules in circumnuclear forms of H. mansonii was fewer than in halteridial forms. By contrast, the number of granules increased as gametocytes of H. meleagridis became circumnuclear.

Haemoproteus stableri can be distinguished from H. meleagridis and H. mansoni by the amoeboid margins of mature gametocytes, their significantly fewer pigment granules and the higher host cell nuclear displacement ratio (White and Bennett, 1979; Greiner and Forrester, 1980).

The strong morphological similarities between haemoproteids of Grouse and H. meleagridis may be significant. Bennett (1960) suggested that the host behavior, habitat and community may be more important in determining the host range of avian haemoproteids than specificity of the parasites themselves. Since the distribution of Ruffed Grouse coincides with that of the Wild Turkey throughout the Appalachian Mountains of the eastern U.S., transmission of H. mansoni and H. meleagridis between either host may be possible. Fallis and Bennett (1960) reported the sporogony of H. mansoni in species of Culicoides from Canada. It is likely that species of Culicoides are vectors of H. mansoni throughout the range of its host. Studies of the experimental cross transmission of H. meleagridis and H. mansoni between Ruffed Grouse and turkeys are needed to determine the validity of both species of Haemoproteus.

The results of the morphometric analysis of H. meleagridis in turkeys, the Ring-necked Pheasant and the Chuckar demonstrate that few significant changes in

parasite morphology occurred. The discriminant analysis of parasite and host cell variables was unable to correctly classify scores from macrogametocytes, microgametocytes and host cells infected with macrogametocytes to their respective host species. This indicates that the gametocytes of H. meleagridis and their associated changes in host cells were essentially identical in each of the 3 host species. The discriminant analysis was more successful in classifying host cells infected with microgametocytes and correctly identified 100% of the Ring-necked Pheasant scores. This was probably related to the much greater lateral displacement of the host cell nucleus that occurred in host cells of this species.

Morphometric studies of species of Leucocytozoon have also failed to show significant morphological variation in gametocytes of a single species in different species of hosts (Bennett and Campbell, 1975; Greiner and Kocan, 1977). These findings led Bennett and Campbell (1975) to synonymize a number of morphologically similar species reported from the same host family. These synonymies were based on experimental evidence that most species of Leucocytozoon are specific to host family and illustrate the importance of studies of cross-transmission. Bennett and Campbell (1975) found that gametocytes of L. dubreuilii, L. fringillinarum and the round gametocytes of L. simondi could not be separated

on the basis of measurements alone. All 3 species were retained because experimental studies had demonstrated their specificity to particular host families. In view of the recent revisions in avian taxonomy, similar studies of host specificity of avian haemoproteids, e.g. H. meleagridis and H. mansonii, should be conducted before synonymies are proposed.

Fine Structure

Mature Gametocytes

Mature gametocytes of H. meleagridis were similar in fine structure to gametocytes of other species of Haemoproteus. Ultrastructural features of the cytotone, nucleus and nucleolus, mitochondria, osmiophilic bodies, cytoplasmic ribosomes and endoplasmic reticulum were essentially identical to gametocytes of H. columbae, H. velans and H. metchnikovi (Bradbury and Roberts, 1970; Sterling, 1972; Sterling and Aikawa, 1973; Bradbury and Trager, 1968a; Dessler, 1972a).

Differences from other species of Haemoproteus were apparent in the pellicle of H. meleagridis. Studies of gametocytes of other species have shown that the pellicle is composed of 3 layers - 2 outer unit

membranes and a thickened, osmiophilic inner membrane composed of 2 unit membranes in close apposition to one another (Bradbury and Roberts, 1970; Sterling, 1972; Sterling and Aikawa, 1973). By contrast, the inner membrane of mature gametocytes of H. meleagridis consisted of a single unit membrane that was thicker and more osmiophilic than the outer 2 membranes. It is unlikely that the difference was a result of poor fixation, since other membranous structures within the gametocytes and their host cells were well preserved.

Sterling and Aikawa (1973) suggested that the double inner membrane of gametocytes of H. columbae was a remnant of the intramembranous complex of merozoites. Based on observations of Plasmodium spp., they suggested that this 2-layered complex was retained in merozoites that had recently invaded a red blood cell if the merozoite was to become a gametocyte. Dedifferentiation of the complex occurred if asexual, schizogonic stages developed. The similarities in structure and development among most haemosporidian parasites make it unlikely that the thickened, inner pellicle of H. meleagridis had a different origin. Perhaps the 2-layered intramembranous complex of merozoites of H. meleagridis fused to form the single, thickened layer that was observed in gametocytes.

Other studies of macrogametocytes of Haemoproteus and Leucocytozoon have noted the presence of amorphous,

moderately electron dense material within the dilated cisternae of the endoplasmic reticulum (Sterling and Aikawa, 1973; Bradbury and Roberts, 1970). Desser et al. (1970b) suggested that it was the precursor to the crystalloid material observed in ookinetes, oocysts and sporozoites of L. simondi. The continuity between the endoplasmic reticulum and the inner, osmiophilic layer of the pellicle of macrogametocytes of H. meleagridis, indicates that this material may also play some role in the changes in pellicular structure that occur prior to their release from the host cell. Other workers have suggested that the osmiophilic bodies may have a similar function and aid in dissolving the host cell membrane during gametogenesis (Aikawa et al., 1969; Rudzinska and Trager, 1968).

Gametogenesis

Early changes in the fine structure of gametocytes of H. meleagridis during the initial stages of gametocyte maturation were identical to changes observed in maturing gametocytes of H. columbae, H. metchnikovi and H. velans (Bradbury and Trager, 1968a; Bradbury and Trager, 1968b; Sterling, 1972; Aikawa and Sterling, 1974a; Desser, 1972a). In all species studied to date, the gametocytes round-up within their host cells. Soon afterward, the outer layer of the pellicle detaches from the outer surface

of the gametocyte in sheets and whorls and floats free in the host cell cytoplasm. Sterling (1972) did not observe the detachment of the outer layer from gametocytes of H. metchnikovi, but did note that it was missing once the gametocytes became extracellular. Desser (1972a) described multilaminar, membranous structures external to maturing, intracellular gametocytes of H. velans, but failed to associate their appearance with the loss of the outer layer of the pellicle in extracellular gametocytes. He suggested instead that they were altered bands of microtubules from host red blood cells that normally aid in maintaining the shape of the cell. Micrographs published by Bradbury and Roberts (1970) and Aikawa and Sterling (1974), as well as observations from this study, leave little doubt that these membranous sheets and whorls originated from the outer layer of the gametocyte pellicle. This process appears to be limited to the genus Haemoproteus and has not been reported from studies of Plasmodium or Leucocytozoon (Aikawa and Sterling, 1974a). Ribosome-like granules similar to those observed on some membranous whorls in this study were also observed by Bradbury and Roberts (1970) on whorls that detached from gametocytes of H. columbae. The significance of the granules and their origin are unknown.

Macrogametogenesis. Few detailed ultrastructural observations of the maturation of macrogametes have been made for any species of Haemoproteus. Bradbury and Trager (1968a) provided the most detailed description. During the initial steps of gametocyte maturation in H. columbae, they noted the detachment of the endoplasmic reticulum from the nuclear envelop and the elongation of the macrogametocyte nucleus from a spherical to an ellipsoidal shape. The changes in nuclear shape were accompanied by the appearance of an intranuclear spindle that converged on an electron dense plaque in the nuclear membrane. Two "atypical" centrioles composed of a circle of 9, single microtubules rather than the conventional circle of 9, triple microtubules were embedded in an electron dense material. They appeared in the cytoplasm next to the electron dense plaque. Approximately 10 minutes after the start of gametocyte maturation, the nucleus constricted twice to form 2, detached maturation bodies. The intranuclear spindle as well as the nucleolus were retained by the nucleus.

A similar process appeared to occur in maturing macrogametocytes of H. meleagridis, although observations are limited. Unfortunately, gametocyte maturation was not followed longer than 3 minutes, so the formation of maturation bodies was not observed. Atypical centrioles were not found in association with the

electron dense thickening and associated intranuclear spindle in 1 macrogametocyte. Since serial sections were not cut, it is likely that they may have been missed.

A single cytostome normally occurs in mature gametocytes of H. columbae and H. metchnikovi (Bradbury and Roberts, 1970; Sterling, 1972). Gallucci (1974a) and Bradbury and Trager (1968a) reported "internal cytostomes" and a single, non-functional peripheral cytostome within mature and immature macrogametes of H. columbae. The internal cytostomes consisted of 2 electron dense rings, but were located in the interior of the cell rather than in the external pellicle. Other observations of cytostomes in maturing macrogametes and exflagellating microgametocytes have not been reported. The presence of cytostomes in developing gametes of H. meleagridis indicates that they persist as part of the pellicle throughout development of the gametes. However, their small size and the absence of associated food vacuoles indicates that they are non-functional.

Microgametogenesis. A number of studies of the microgametogenesis of avian haemoproteids have been conducted. Bradbury and Trager (1968a, 1968b), Sterling (1972) and Aikawa and Sterling (1974a) studied H. columbae and H. metchnikovi. They reported the development of axonemes composed of 9 peripheral microtubules that

surrounded 2 central microtubules during the earliest stages of exflagellation. These were located free in the cytoplasm in various stages of assembly. One end of developing axonemes was usually associated with a dense plaque in the nuclear membrane that usually had associated intranuclear tubules. Sterling (1972) reported an atypical centriole embedded in electron dense material adjacent to the plaque. He observed the attachment of axonemes to basal bodies that were associated with the atypical centriole. Aikawa and Sterling (1974a) found that the intranuclear microtubules extended across the nucleus of exflagellating gametocytes of H. columbae to another electron dense plaque on the opposite side of the organelle. They observed the condensation of electron dense material around the base of the plaques and suggested that this material was eventually incorporated into the microgamete nucleus. As axonemes began to bud from the exterior of microgametocytes, Aikawa and Sterling (1974a) described the protrusion of a portion of the microgametocyte nucleus to the base of the bud. They presented micrographs indicating that a portion of the nucleus, still bound by a nuclear membrane, detached from the microgametocyte nucleus and became incorporated into the exflagellating microgamete as a spiral around the axoneme. Sterling (1972) described a similar process during the exflagellation of H. metchnikovi.

Bradbury and Trager (1968b) described the polarization of microgametocytes of H. columbae into 2 halves - 1 that contained organelles such as axonemes, mitochondria and food vacuoles and another that contained remnants of the microgametocyte nucleus. They observed the disintegration of the nuclear membrane and a condensation of nuclear material around the bases of developing axonemes. The condensed masses of nuclear material were subsequently surrounded by a membrane and incorporated into microgametes that "peeled" from the microgametocyte.

Desser (1972a) described the formation of atypical centrioles and electron dense nuclear plaques in microgametocytes of H. velans. He did not observe the polarization of exflagellating microgametocytes described by Bradbury and Trager (1968b), but did describe the breakdown of the nuclear membrane and the subsequent condensation of masses of chromatin around the bases of developing axonemes.

Observations from this study are very limited, but they are most similar to the process described by Aikawa and Sterling (1974a). The nuclear membrane remained intact in microgametocytes of H. meleagridis throughout the process of exflagellation, but was often difficult to discern. During the final budding of microgametes, the nucleus appeared to be stretched to their base. The

polarization of microgametocytes of H. meleagridis into 2 halves was not observed. Atypical centrioles were not detected, but they may have been missed since serial sections were not cut.

Microgametes of H. meleagridis were similar to microgametes of other species of Haemoproteus. Microgametes of most other species contain a single axoneme and a small, membrane-bound nucleus. Mitochondria have not been observed (Aikawa and Sterling, 1974a; Sterling, 1972; Desser, 1972a). Bradbury and Trager (1968a) observed 2 axonemes in microgametes of H. columbae, but these observations have not been confirmed (Aikawa and Sterling, 1974a). The periodic striations observed in the central microtubules of axonemes of H. meleagridis have been reported in axonemes of H. metchnikovi and L. simondi (Sterling, 1972; Aikawa et al., 1970).

Oocysts

Most ultrastructural studies of the oocysts of haemosporidian parasites have been limited to avian and mammalian species of Plasmodium (Mehlhorn et al., 1980; Sinden and Strong, 1978; Canning and Sinden, 1973; Howells and Davies, 1971; Terzakis, 1971; Terzakis et al., 1966; Duncan et al., 1960) and to 3 species of Leucocytozoon (Desser and Allison, 1979; Wong and Desser, 1976; Desser, 1972c). In spite of the importance of comparative

studies in elucidating the taxonomic and phylogenetic relationships among the Haemosporina, only 1 study of the oocysts of Haemoproteus has been published (Sterling and DeGiusti, 1974). These workers found that H. metchnikovi, a chelonian parasite that develops in deer flies, shares features of its sporogonic development with Plasmodium and Leucocytozoon that neither of these 2 genera hold in common.

Differentiation of the oocyst. The sporogony of avian and mammalian species of Plasmodium follows a number of similar steps (Mehlhorn et al., 1980; Sinden and Strong, 1978; Garnham et al., 1969; Terzakis et al., 1967; Terzakis et al., 1966; Duncan et al., 1960). After penetrating the midgut, the ookinete rounds-up under the basement membrane. Mehlhorn et al. (1980) found the remains of polar rings and micronemes derived from the apical complex of the ookinete at the periphery of early P. gallinaceum oocysts. Similarly, Garnham et al. (1969) found remnants of this complex in a dimple or small groove in the peripheral cytoplasm of early oocysts of P. berghei yoelii. Studies of older Plasmodium oocysts have failed to find evidence that these organelles persist more than a few days. Among other Haemosporina, the remnants of apical organelles from the ookinete have been observed in early, but not later oocysts of L. tawaki (Desser and

Allison, 1979). Sterling and DeGiusti (1974) observed these organelles throughout the development of oocysts of H. metchnikovi and in the residual body after sporozoite differentiation was complete. Apical organelles derived from the ookinete of H. meleagridis appear to persist in the cytoplasm throughout the life of the oocyst. There is no evidence that any of them are reused in the formation of sporozoites (Garnham et al., 1969).

The early stages of sporozoite formation in Plasmodium are initiated by vacuolization of the peripheral oocyst cytoplasm. This process also occurs in early oocysts of H. metchnikovi, but has not been described in any of the 3 species of Leucocytozoon that have been studied. Terzakis et al. (1966) suggested that the vacuoles may develop after a local change in ionic concentration in the peripheral cytoplasm causes water to cross the oocyst wall or, alternatively, after the oocyst cytoplasm secretes fluid at the oocyst periphery. In support of the latter, Sinden and Strong (1978) described the formation of vacuoles in oocysts of P. falciparum by the fusion of membranous vesicles that originated in the oocyst cytoplasm.

Electron dense, linear thickenings appear under the membrane soon after vacuolization begins. The vacuoles eventually coalesce to form cytoplasmic clefts which subdivide the oocyst cytoplasm. Sinden and Strong (1978) found that clefts in the oocysts of P. falciparum

originated from endoplasmic reticulum scattered in the cytoplasm and from peripheral vacuolization of the oocyst. Clefts originating from the endoplasmic reticulum were covered on their cytoplasmic face by additional 2-layered membranous sacs. These were lined on their innermost face by a thin amorphous electron-dense coating analogous to the electron, linear thickenings described under the plasma membrane of other species.

As subdivision of the cytoplasm continues, budding sporozoites develop under the electron dense thickenings. Apical organelles including polar rings, micronemes and subpellicular microtubules differentiate as the sporozoites bud from the sporoblast body. In contrast to Plasmodium, H. metchnikovi does not undergo cleft formation. Instead, budding sporozoites develop around the periphery of the central sporoblast body (Sterling and DeGiusti, 1974).

The differentiation of early oocysts of Leucocytozoon occurs without vacuolization and cleft formation. As sporozoites bud from electron dense thickenings under the peripheral plasma membrane, the sporoblast body slowly contracts (Desser and Wright, 1968; Desser, 1972c; Wong and Desser, 1976; Desser and Allison, 1979). Sporozoite differentiation and budding resembles Plasmodium. Leucocytozoon oocysts are smaller and develop fewer sporozoites than Plasmodium oocysts. The cleft formation,

subdivision of the oocyst cytoplasm into many sporoblast bodies and the endogenous or internal sporozoite budding that occurs in Plasmodium is probably an adaptation to increase the surface area available for sporozoite formation (Sinden and Strong, 1978).

Limited observations of Il. meleagridis from this study suggest that vacuolization and cleft formation do not occur. Thus, this parasite closely resembles Leucocytozoon in size of oocysts and number of sporozoites produced as well as in contraction of the sporoblast body and differentiation of the sporozoites.

Nuclear divisions. The nuclear events following fertilization of haemosporidian parasites are still poorly understood. Bano (1959) and Canning and Anwar (1968) found evidence of post-zygotic meiosis in the early oocysts of Plasmodium. They observed what they believed to be 4 chromosomes in diploid oocysts and 2 in haploid oocysts in stained preparations. Later studies with electron microscopy have shown that the chromosomes do not condense. The dark-staining nuclear masses observed earlier may have been nucleoli (Mehlhorn et al., 1980) or fragments of an enlarged digitate nucleus (Canning and Sinden, 1973). Mehlhorn et al. (1980) observed nuclear spindles in zygotes and in ookinetes of P. gallinaceum and

suggested that zygotic rather than post-zygotic meiosis had occurred.

Attempts to trace division of the nucleus during differentiation of Plasmodium oocysts have been only partly successful. This is largely because of the difficulty in obtaining and interpreting serial sections through the complex, multidigitate form of the nucleus. A number of investigators have found that nuclear division proceeds without the disappearance of the nuclear membrane. Electron dense masses termed centriolar plaques (Howells and Davies, 1971), kinetic centers (Schrevel et al., 1977) and spindle pole bodies (Kubai, 1975) appear in cup-like invaginations of the nuclear envelope. These are duplicated throughout the nuclear envelope and direct a series of multiple, asynchronous mitotic divisions. Each kinetic center is linked with another by a thin band of dense material. Radiating from each are spindle microtubules of different lengths. The shorter ones appear to be attached to electron dense kinetochores. These are believed to be attached to individual chromosomes (Schrevel et al., 1977). Schrevel et al. (1977) counted 8 kinetochores at each halfspindle pole, suggesting that the haploid chromosome number in P. berghei may be as few as 4. Canning and Sinden (1973) estimated that the haploid number could be as few as 5 and possibly as high as 10 from kinetochore counts on their micrographs of P. berghei.

Since most studies of oocysts of haemosporidian parasites are not based on serial sections, it is not clear when final fragmentation of the large polyploid nucleus occurs. Howells and Davies (1971) suggested that the large, lobulate nucleus of P. berghei may break up soon after cleft formation subdivides the cytoplasm into sporoblast bodies. Each nucleus may then undergo a final division as opposing ends migrate into sporozoite buds. Schrevel et al. (1977) felt that budding and fragmentation of the nucleus occurred at the same time sporozoites budded from the sporoblast body. They also suggested that the multiple mitoses that occurred prior to sporozoite differentiation allowed numerous genetic units to be positioned in the nuclear cortex for the direction of sporozoite differentiation.

Studies of Leucocytozoon have indicated that nuclear division may occur in a manner similar to Plasmodium. Multiple kinetic centers have been observed in the nuclear envelope of L. simondi, L. dubreuilii and L. tawaki, but the sequence of events leading to final fragmentation of the nucleus has not been studied in detail (Desser, 1972c; Wong and Desser, 1976; Desser and Allison, 1979).

Kinetic centers have not been described in H. metchnikovi, nor were they found in this study. However, it seems likely that haemoproteids may undergo

nuclear divisions in a manner similar to Plasmodium and Leucocytozoon.

Crystalloid. Crystalloid aggregations of electron dense particles consisting of a lipo-protein complex have been reported from the oocysts of Leucocytozoon, H. metchnikovi and from the early oocysts of P. gallinaceum and P. berghei (Trefiak and Desser, 1973). Trefiak and Desser (1973) found that they originate from amorphous aggregations of electron dense material in the macrogametes of L. simondi. They suggested that the crystalloid functions as an energy source since it appears to be utilized quickly in rapidly growing oocysts of Plasmodium and is incorporated into the sporozoites of Leucocytozoon and H. metchnikovi. The former have been found to persist in their avian hosts for up to 11 days and may require a reserve energy source to maintain their viability (Khan et al., 1969).

Membrane-bound lipid inclusions have been reported in the oocysts of P. cynomolgi, P. gallinaceum, P. falciparum, L. simondi, L. dubreuilii and L. tawaki (Terzakis, 1971; Terzakis et al., 1966; Sinden and Strong, 1978; Desser, 1972c; Wong and Desser, 1976; Desser and Allison, 1979). They were not reported in oocysts of H. metchnikovi (Sterling and DeGiusti, 1974). Generally, lipid inclusions

tend to be smaller in oocysts of those species that form prominent crystalloids.

The absence of a crystalloid in 3-day-old and 6-day-old oocysts of H. meleagridis suggests that the large lipid-like inclusions observed in this study may function as energy reserves. Observations of earlier oocysts are needed to determine whether a crystalloid is present in this parasite.

Megaloschizonts

The thick laminated cyst wall and the process of merozoite formation within mature megaloschizonts of H. meleagridis appears to be unique among haemosporidian parasites. Observations of megaloschizonts of L. simondi and the megaloschizonts of the unidentified haemosporidian from Northern Bobwhites are most similar to those of this study. Dessler (1970a) reported a capsule around megaloschizonts of L. simondi composed of a meshwork of reticular fibers and an outer fibrous layer. The capsule was external to the host cell plasma membrane. Dessler and Fallis (1967) suggested that it was primarily of host origin and probably secreted by fibroblasts that were often found surrounding the megaloschizonts and their host cells. Gardiner et al. (1984) found that megaloschizonts of the parasite of Northern Bobwhites were surrounded by a moderately dense, amorphous wall that

had villous processes extending from their external surfaces. They also noted that the megaloschizonts were divided into thick-walled compartments resembling tubes.

Megaloschizonts of H. meleagridis differ from those observed by Desser (1970a) and Gardiner et al. (1984). The megaloschizonts observed in this study were extracellular and had thick, laminated walls composed of electron-dense, granular material. Material examined by Gardiner et al. (1984) was fixed initially in buffered formalin and then post-fixed with osmium-dichromate and processed for electron microscopy. The poor preservation of cellular detail in their samples make comparisons with the megaloschizonts of H. meleagridis difficult. However, the villous processes on the cyst walls and compartmentalization of megaloschizonts by the thick wall were never observed in megaloschizonts of H. meleagridis.

Desser (1970a) described merozoite formation in megaloschizonts of L. simondi by fragmentation of the cytomere cytoplasm. By contrast, Gardiner et al. (1984) reported that merozoite development in megaloschizonts from Northern Bobwhites occurred by budding into an interior vacuole within the cytomere cytoplasm rather than by the protrusion of developing merozoites from the cytomere surface. Neither process was observed in megaloschizonts of H. meleagridis.

Mature merozoites of H. meleagridis are very similar to merozoites of other haemosporidian parasites. All have a specialized anterior end containing 3 polar rings, a pair of electron dense rhoptries and small micronemes (Aikawa and Sterling, 1974b). Cytostomes have been reported in merozoites of Plasmodium and Haemoproteus, but have not been observed in merozoites of species of Leucocytozoon (Aikawa and Sterling, 1974b). They were not observed in merozoites of H. meleagridis, but observations are limited. Other organelles, including the nucleus, mitochondria and pellicle, were similar to merozoites of other haemosporidian parasites (Aikawa and Sterling, 1974b).

The large, electron-lucent vacuoles observed in mature merozoites of H. meleagridis have not been reported from other haemosporidian merozoites (Figures 90, 91). The vacuole persists during development of the early gametocytes and is clearly visible in Giemsa-stained blood films. It disappears by the time gametocytes reach maturity. The function and origin of this organelle are unknown.

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BIOGRAPHICAL SKETCH

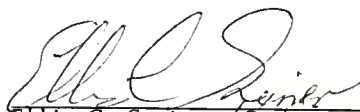
Carter Tait Atkinson was born on November 23, 1954, in Bethesda, Maryland. He graduated from Gaithersburg High School in June, 1972, and received a Bachelor of Science degree with honors in biology from Dickinson College in May, 1976. During the following 3 summers, he worked as a field ornithologist for the U.S. Fish and Wildlife Service on their Hawaii Forest Bird Survey. While working in the Hawaiian Islands, he became interested in the role of introduced diseases in the decline and extinction of native Hawaiian birds. This interest led him to enroll as a graduate student at the University of Maryland between September, 1978, and May, 1979. In August, 1979, he enrolled as a student in the Department of Tropical Medicine and Medical Parasitology at the Louisiana State University Medical Center in New Orleans. He graduated in August, 1981, and received a Master of Science degree in parasitology. In September, 1981, he enrolled in the graduate program at the University of Florida.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



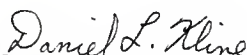
Donald J. Forrester, Chairman
Professor of Veterinary Medicine

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Daniel L. Kline
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This dissertation was submitted to the Graduate Faculty of the College of Medicine and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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